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BY

DR. FRANZ LAFAR,

Professor of Fermentation-Physiology and Bacteriology in the Imperial Technical High School, Vienna.

TRANSLATED BY CHARLES T. C. SALTER.

VOL. II.—**EUMYCETIC FERMENTATION.**

PART I.

With 68 Figures in the Text.

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PUBLISHERS' NOTE.

[*For preface to the work, written by Dr.* Emil. Chr. Hansen, *see volume I.*]

Since the publication of the first volume of this important work we have been repeatedly asked for the companion volume. The German publishers have now arranged to issue the second volume in parts rather than await the completion of the work. Advance sheets have therefore been placed in our hands, and we are now able to provide English readers with the present instalment, which constitutes Part I. of the second volume.

The concluding portion of the volume will follow as soon as the German proofs come to hand.

January 1903.

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TECHNICAL MYCOLOGY.

SECTION X.

RUDIMENTS OF THE GENERAL MORPHOLOGY AND PHYSIOLOGY OF THE EUMYCETES.

CHAPTER XXXIX.

GENERAL MORPHOLOGY.

§ 217.—Articulation of the Thallus.

ALREADY in the first volume (§ 22) the algæ and the fungi were arranged in a single group, that of the **Thallophytes**, in contradistinction to all other plants, the latter being classed in the group of **Cormophytes**. The distinguishing characteristics of these two groups were stated to be the absence in the former, and the presence in the latter, of an articulation of the body of the individual organism into leaf and stem. At the same time, it was mentioned that the corporeal form assumed by the *Thallophytes*, and differing fundamentally from the **cormus** of the *Cormophytes*, has received the general name of **thallus**. That intermediate forms between these two types should exist, and that the thallus of the highest *Thallophytes* should approximate to the cormus of the lowest *Cormophytes*, is perfectly natural and in accordance with the general laws of phylogenetic evolution.

Although the fungi, the only class of *Thallophytes* with which we are now concerned, do not exhibit division into leaf and stem, their thallus is not entirely destitute of all articulation. True, in one of the two chief divisions of the fungus family, the **Schizomycetes**, the articulation of the thallus is practically undiscernible, the individual organisms taking the form of globular or oval cells, or straight or bent rods of variable length. Should any extensive development of the thallus occur, this may almost invariably be regarded as either a malformation preceding death—*e.g.* the branching of bacteroids (§ 195) and *Bacterium aceti* (§ 211)—or as an assemblage of several individual organisms giving rise to a deceptive appearance of articulation, as in the case of *Cladothrix* (§ 197). Again, in many species of bacteria, the colonies known as zoogloœa seem to exhibit a more or less well-developed articulation; but these cannot be con-

sidered as a thallus, since they represent assemblages of many uniform cells, and not separate individual organisms.

The case is, however, different in the second division of the fungus family (§ 23), namely, the **Eumycetes** or higher fungi. These differ from the *Schizomycetes* in universally possessing the faculty of forming **true branchings.** This characteristic resides in the immediate and uninterrupted connection between the plasma of the branch (or the oldest cell of same) and that of the main stem from which the branch proceeds. The divergence of form and the luxuriance of this branching vary in the different orders of fungi, and, in general, increase the higher we

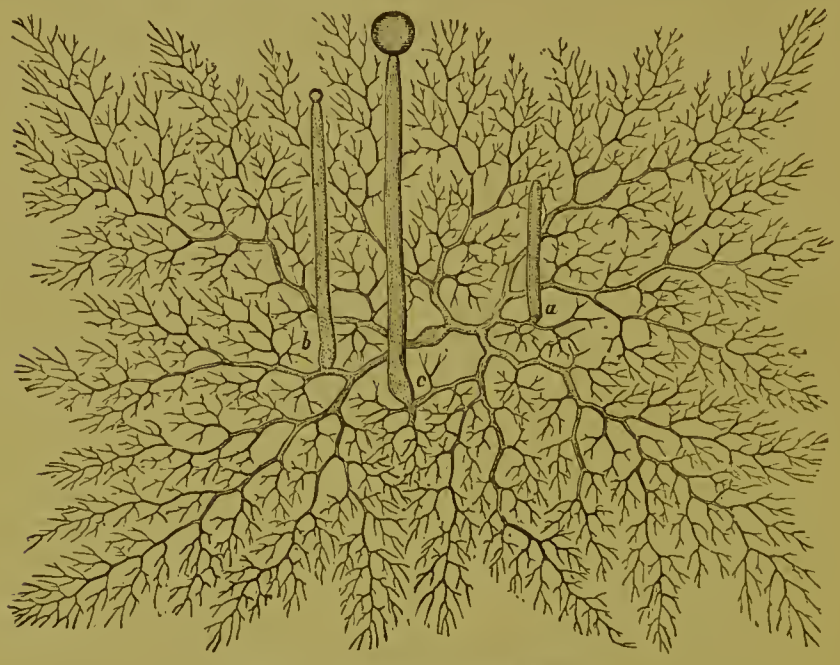


FIG. 91.—Thallus of Mucor mucedo.

Shows the unicellular mycelium sprung from the spore; together with three organs of fructification, *a*, *b*, *c*, in different stages of development, raising themselves from the mycelium. Magn. about 10. (*After Kny.*)

get in the system and the nearer we approach the *Cormophytes*. In the lowest members, on the other hand, this tendency is often greatly simplified and restricted, thus approximating to the *Schizomycetes*.

A more careful examination of the thallus of the higher *Eumycetes*, even with the unassisted eye, will reveal something more than the existence of a more or less copious branching. It will soon be found possible to dissect the thallus into two parts (Fig. 91), which though intimately connected serve entirely different purposes: one, known as the **mycelium**, having charge of the nutrition and maintenance of the individual plant; whilst, on

the other, or **organ of fructification**, devolves the task of reproduction, and therefore the maintenance of the species. This latter organ develops special cells or **spores**, which are mostly globular or oval, and from each of which under favourable circumstances, a new individual of the same species can be produced.

The *mycelium* may therefore be defined as the portion of the thallus spreading in or upon the nutrient medium and extracting nutriment therefrom. It proceeds from a spore. As soon as this latter comes under the influence of circumstances favourable to its germination, it absorbs water and other nutrient materials from the surroundings, swells up more or less, and usually puts forth one or more **tubular buds** (Fig. 92). These continue to develop in two directions, increasing in length, and forming lateral branches which in turn continue to act in like manner The name **hypha** is given to each of these branchings, and the whole group of *hyphæ* that have resulted from a single spore and serve to nourish the individual plant in question, is called the *mycelium*. The spore may also germinate by the process of gemmation described in § 219. In some fungi the spores can only germinate in the one manner, whilst in others the germination is restricted to the other type. The mycelium, in order to fulfil its task, must continue to penetrate towards more remote portions of the nutrient medium; and accordingly, the hyphæ must progressively increase in length. Now this growth is confined to the apex of the hyphæ, *i.e.* the part farthest from the centre of development. On the other hand, the parts nearest that centre quickly cease to extend and branch; consequently, the *Eumycetes* exhibit acrogenous growth. This behaviour constitutes another feature of difference between *Eumycetes* and *Schizomycetes*, since, in the latter, the growth of the cells is not restricted to one end only, but proceeds by the extension of the whole body.

FIG. 92.—Mucor mucedo. Germinating spore, which has already put forth two buds. Magn. 300. (*After Brefeld.*)

Examination of the mycelia of a large number of species of *Eumycetes*, under a power of about 100 diameters, soon leads to a separation of these specimens into two groups: the one comprising species whose mycelium, however large and extensively branched, consists of only one single cell; whilst the other group contains the species wherein the mycelial hyphæ are subdivided into cylindrical parts of variable length by transverse walls (**septa**) perpendicular to the longitudinal axis. This fundamental and highly important difference constitutes the chief basis for the separation of the *Eumycetes* into two main subdivisions: *Eumycetes* with a unicellular mycelium on the one hand, and *Eumycetes* with a septated mycelium on the other.

In mycelial structure and several other particulars, the members of the first subdivision bear a remarkable resemblance to certain unicellular algæ, for which reason they have received the name **Phycomycetes**, or *algic fungi*. On the other hand, the *Eumycetes* of the second group, with septated mycelia, bear the group-name **Mycomycetes**. In comparison to the others these latter stand on a higher plane of development, and are almost exclusively aerial; whereas the majority of the *Phycomycetes* still incline to subaqueous existence. The following scheme will easily fix the foregoing classification in the reader's memory :—

- Vegetable Kingdom
  - *Cormophyta*
  - *Thallophyta*
    - *Algæ* (producing chlorophyll)
    - *Fungi* (*Mycetes*) (not producing chlorophyll)
      - *Eumycetes* (Acrogenous true branchings)
        - *Mycomycetes* (septated mycelium)
        - *Phycomycetes* (unicellular mycelium)
      - *Schizomycetes* (Fission fungi)

§ 218.—The Typical Mycelium.

We will now consider the **development of the mycelium of a Mycomyces from its spore**, Fig. 93 helping to make this clear. Soon after the tubular buds have sprouted from the spore, a septum forms between the spore and each of the buds. The tube then increases in length, and develops in its interior a septum which divides it into two cells, the one nearer the spore (or centre of growth) being termed the **inner cell**, whilst the outer one is called the **terminal** or **crown cell**. Now, whereas the inner cell ceases to increase in length the crown cell continues to grow longitudinally, and in turn develops a septum, whereby an inner cell (of the second order) is again formed; and this operation is repeated at convenience. Meanwhile the inner cells are not inactive, since, although they do not increase in length, they throw out lateral projections, which develop into branches separated from the inner cell by septa. These branches grow longitudinally, and separate into a crown cell and a secondary inner cell by developing another septum, an operation repeated by the crown cells as often as external circumstances will admit. This faculty of the inner cells of the first order is also shared by those subsequently formed, so that we have lateral branches of the third, fourth, and other orders. The whole of these hyphæ or mycelial threads together constitute the mycelium. The serial order in which the inner cells begin to throw off lateral branches is, as a rule, in accordance with their age, the oldest starting first. Consequently, the development of the mycelium proceeds laterally from the spore (the "basis") towards the periphery or apex of the mycelial thread, such a mode of growth being termed **basi-**

fugal or acropetalous. Again, the lateral position of the branches in question is, as a rule, very uniform ; those from the inner cells of odd-numbered orders all branching from one (*e.g.* the left) side, and all those springing from inner cells of even-numbered orders appearing on the opposite (*e.g.* right) side of the respective inner cells. When the branching from any given

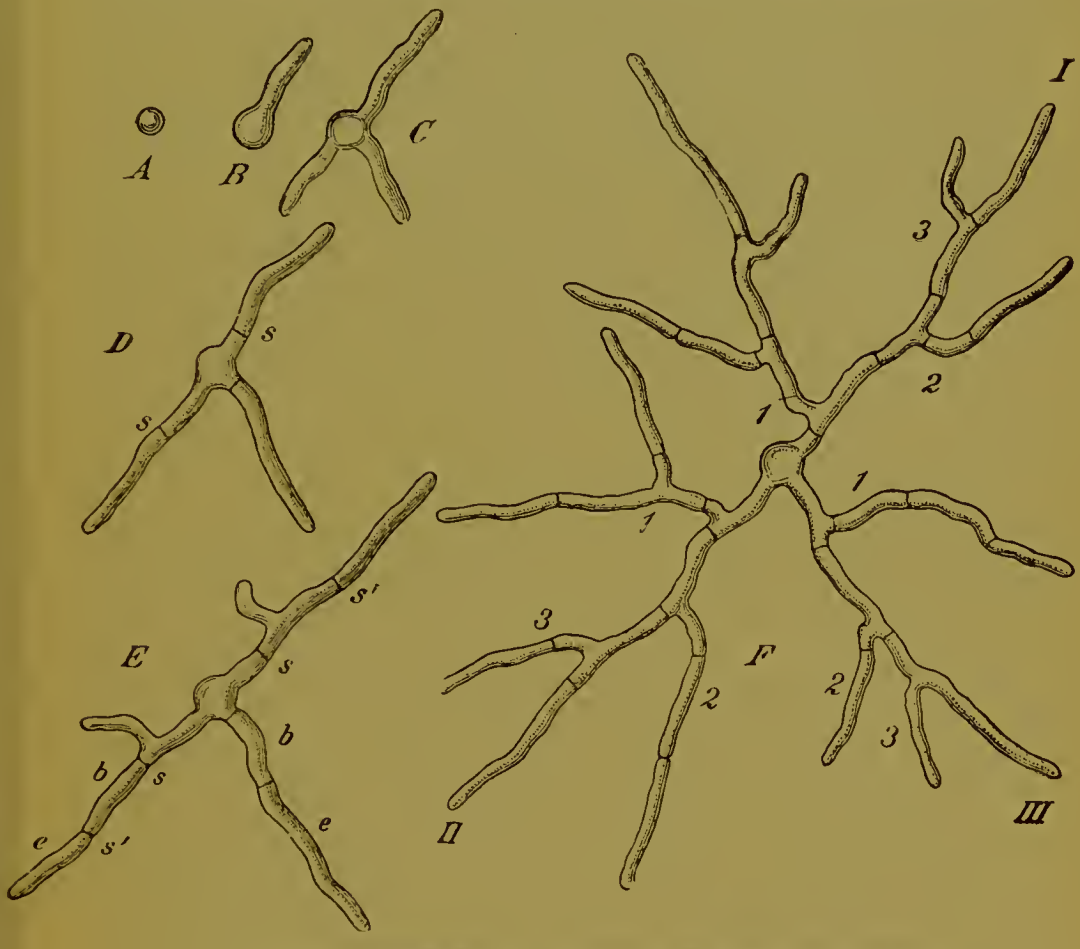


FIG. 93.—Mycelial development of a Mycomyces (the ordinary bread mould, *Penicillium glaucum*).

A, the ripe spore. *B* and *C*, the same, with respectively one and three tubular offshoots. In *D* each of these has become separated from the spore by a septum, *s*. In *E* each tube has become divided by the formation of a second septum, *s'*, into a terminal cell (*e*) and an inner cell (*b*), whilst branching has commenced in two places. *F* shows each of the three tubes developed into a main branch (I. to III.), which in turn has thrown out lateral branches of the first to the third order (1, 2, 3). Magn. 400. (*After Zopf.*)

cell is confined to a single lateral offshoot the system is termed **monopodial**, the main and branch cell together being called a **monopodium**.

The progress of mycelial development in a *Phycomyces* naturally differs from that just described, inasmuch as no septa are

formed under normal circumstances; consequently there is, in this case, no separation of the elongating tubular bud into inner and crown cells. In such event the resulting mycelium consists, as already stated, of a single, many-branched, tubular, non-septated cell, such as is shown in Fig. 91.

The foregoing statement that the inner cells do not play any further part in the mycelial growth of the *Mycomycetes*, inasmuch as they neither extend in length nor develop septa, may be taken as the rule. There are, however, exceptions, septation, accompanied by elongation, frequently occurring within the inner cells in the event of abnormal conditions of nutrition. This phenomenon is termed **intercalary growth**, or **intercalary septation**, to distinguish it from **acrogenous growth**.

If, in the absence of external causes of hindrance, the growth of the mycelium is able to proceed equally in all directions, a stellar system of radial, branched threads, with the spore as a centre, is the result. This form of growth was termed a **typical mycelium** by Zopf. The practical worker in a mycological laboratory can obtain such typical mycelia in a youthful condition, and consequently easy to survey, if he re-examines, after a lapse of one or two days, the plate cultures (§ 85) that have already been examined for the purposes of mycological analysis (*e.g.* of water, milk, beer). During the first investigation the spores of all kinds of mould fungi from the air will have settled on the solid nutrient medium, each of them then germinating to form a mycelium, and thus yielding, as it were, a self-prepared culture.

Mention must here be made of one of the various instances of irregular mycelial development, since it will have to be referred to on a subsequent occasion: this is the phenomenon of intergrowth. It is caused by one of the cells in a mycelium putting forth a branch into the interior of an adjoining cell, so as to displace the intervening septum. The invader may then become divided into cells within the plasma of the invested cell, with the result that an inexpert observer may easily be led to believe that endogenous spores are present. An example of this growth is represented in Fig. 94. Another will be found in a later section dealing with *Dematium pullulans*, and a third in the case of *Oidium Ludwigii Hansen*, occurring in mucilaginous discharges from trees (§ 248), and investigated by W. Holtz (I.). This was probably also the method of formation of the alleged spores observed by Edm. Kayser (V.) within the hyphæ of an unknown mycelial fungus isolated by him from fermenting pine-apple juice. Intergrowths also occur in the sporangia of several fungi.

In the case of a large number of fungi, the development of the mycelium ceases with the formation of the branched hyphæ, the ensuing process being the elaboration of organs of fructifica-

tion. Fungi exhibiting this class of simple mycelial structure are classed under the generic name **Hyphomycetes** or **Thread Fungi**. The term **Mucedinæ**, occurring in the French and English literature, expresses about the same thing. It may be remarked in passing that several botanists, *e.g.* Strasburger, Noll, Schenck, and Schimper, in their botanical text-book, employ the name *Hyphomycetes* in a far wider sense, namely, to include the whole of the *Eumycetes*, the reason for this being that the production of hyphæ is characteristic of these fungi, and constitutes a fundamental distinction between them and the other divisions of the fungus family, the *Schizomycetes* in particular. Nevertheless, in the following pages we will apply the term in its more restricted sense.

In many of the other classes of *Eumycetes*, the development of the mycelium does not cease at the stage we have described as the typical mycelium, but extends further, to the production of

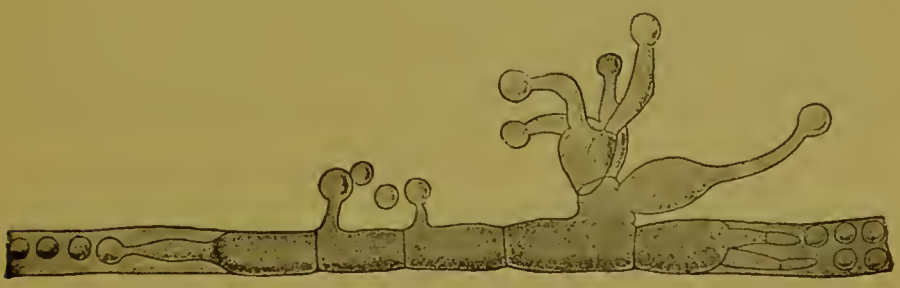


FIG. 94.—Botrytis cinerea.

Intergrowth. Each of the two penultimate cells of the depicted fragment of mycelium has grown into its neighbour, and there become separated into globular cells. The central cell of the mycelial thread has put forth abnormally developed organs of fructification. (*After P. Lindner.*)

aggregations, of the forms known as **mycelial threads** and **mycelial films**. A combination of these two forms constitutes the large bodies known in colloquial language as "mushrooms" or "fungus"; the botanist, however, terming them **fungoid bodies**. The capacity of forming such bodies, upon or within which the organs of fructification are situated, is confined to the most highly developed species of fungi. An example is given in Fig. 95. This, however, is only one variety (though appearing in numerous modifications) of the coalescence and intertwined growth of hyphæ, another form being that of the so-called **sclerotium** or **hard mycelium**. The well-known ergot of rye, which will be more closely described in the last section but one, forms an example of a sclerotium. This is constructed of closely intertwined hyphæ, furnished with a store of nutrient material, and constituting a hard permanent form, which, after a variable period of quiescence, awakens to active life, puts forth organs of

fructification, and is then able to await and utilise the occurrence of favourable conditions, in order to effect the reproduction of the individual from which it has originated. An observation on the artificial production of such permanent forms has been communicated by J. Ray (I.). Among the foodstuffs accumulated in the cells of the sclerotium, special importance attaches to glycogen (§ 253) as the source of easily liberated chemical energy and abundant disengagement of heat. This substance was first observed—without, however, being specially named — by A. de Bary (I.) in the sclerotium of *Coprinus stercorarius;* and it was afterwards found, by W. Rothert (I.), in that of *Sclerotium hydrophilum.* A thin section of sclerotium, or of a fungoid body—both of which are, as already stated, composed of a network of hyphæ—exhibits under the microscope an appearance similar to that of the parenchyma of higher plants, *e.g.* a section through the flesh of an apple. On account of this similarity, these networks of hyphæ have received the name *pseudoparenchyma*, which, however, is not intended to express any further likeness, whether in respect of the mode of formation or physiological purpose. It is perhaps superfluous to emphasise that the mycelia of this class of fungi consist merely of hyphæ when in the earliest stages of existence and consequently at such times are indistinguishable in this respect from the mycelia of the *Hyphomycetes.*

Fig. 95.—Boletus edulis.
Longitudinal section (above) and transverse section (below) through the fruit stem ("fungus") of Boletus edulis. Magn. 300. (*After Strasburger.*)

§ 219.—The Gemmating Mycelium.

The application of the name "typical" to a mycelium growing in the manner described in the preceding paragraph, indicates the possibility of other methods of growth, manifesting themselves as modifications and simplifications of this form. Of these the most important, from our point of view, is the **gemmating mycelium**, the development of which proceeds in the following manner (Fig. 96): The germ cell, or mother-cell, puts forth a protrusion which, however, instead of enlarging to a tube

as in the case of the incipient typical mycelium, assumes a form resembling that of the parent-cell and therefore termed a bud. The daughter-cell then becomes divided from the parent by a septum, which subsequently splits into two layers and enables the two cells to separate. In many instances the parent-cell puts forth only a single bud, but in others two or more. As soon as the daughter-cell has attained the size of the parent, it is then able to behave in turn like the latter, and itself put forth a bud (of the second order), from which again proceeds another bud (of the third order) and so on.

If the parent-cell—as is the case, for example, in most kinds of yeast—be globular, oval, or lemon-shaped, the daughter-cell will also usually be of similar form, and is then termed a short bud. Such globular buds are referred to in the older literature (and occasionally even now) as **spherical yeast**, more particularly in the case of *Mucor*. If, on the other hand, the parent-cell be of elongated form, the daughter-cells issuing therefrom will preferentially develop in a longitudinal direction from the start, and thus form elongated buds. Most of the species of *Mycoderma* afford examples of this type. Fungi with gemmating mycelia of this kind are therefore, in this respect, intermediate to the fungi with typical mycelia.

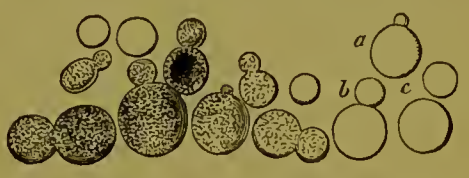


FIG. 96.—Gemmation of a Tortula in Beer wort.

At (*a*) one of the cells has just put forth a tiny bud. At the end of 1½ hours (*b*) this has become considerably larger. After another two hours it has grown to half the size of the parent cell, and has already separated from the latter. Magn. 1000. (*After Hansen.*)

The above-mentioned double stratification of the septa between the cells produced in the foregoing manner, permits these cells to enjoy an independent existence, and consequently enables them to be separated from one another. In many instances this actually occurs, and consequently the nutrient medium wherein this takes place, will exhibit a comparatively large number of single cells. Conversely, in other instances, the successively developed buds remain connected together, forming a cellular aggregation (Fig. 97). In the older literature, such aggregations, when composed of globular cells, and therefore resembling a series of small knots (Lat. *Torula*), were generally named **Torulæ**. This was afterwards employed as the generic name for a number of species, some of which are capable of exciting alcoholic fermentation and will be described in a later section. An example of these is given in Fig. 98.

The form of the gemmæ from one and the same species is also dependent on the temperature and the conditions of nutrition, as has been shown by E. Ch. Hansen in the case of beer yeasts and wine yeasts. These, when submerged in beer wort, develop

mycelia constructed of short gemmæ; whereas, when cultivated on the surface of the liquid, and therefore in presence of abundance of air, they form mycelia composed of elongated buds. Further particulars of this will be found in § 246.

The formation of mycelia composed of gemmæ was first observed in the case of yeast fungi, and was regarded as a method of development peculiar to these organisms. Bail (I.), however, in 1857, showed that this phenomenon also appears in certain species of *Mucor* (see Chapter xliv.) when submerged in

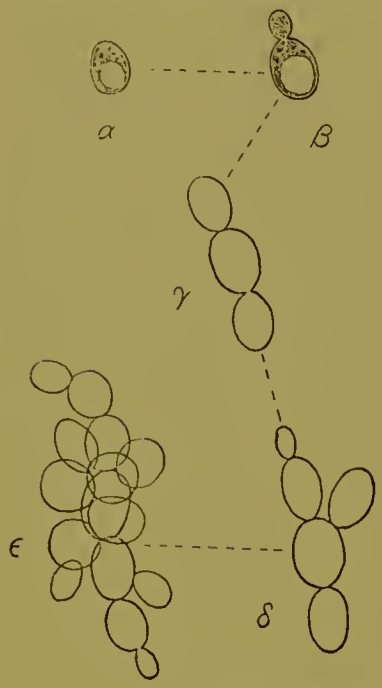


Fig. 97.—Saccharomyces pyriformis Ward.

Cell α, embedded in a hanging drop of gingerbeer gelatin and kept at a temperature of 15° C., threw out a bud (β) within 4½ hours. At the end of another 14 hours three normal cells (γ) were present, which grew to the aggregation δ in another 10 hours. This in turn had developed into the colony ε in 13½ hours more. (*After M. Ward.*)

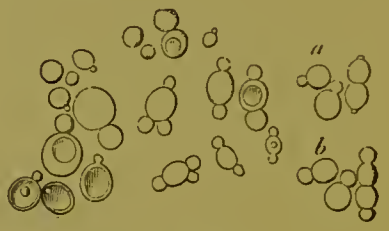


Fig. 98.—Torula.

Specimen grown in beer wort. *a* represents a group of gemmating cells, the condition of which, after the lapse of an hour, is shown at *b*. Magn. about 1000. (*After Hansen.*)

a nutrient solution containing sugar. For more precise observations of this phenomenon in the case of *Mucors*, we are indebted to Brefeld (XVI.). With *Mucor racemosus*, the carbon dioxide collecting in the nutrient fluid acts upon the cells, by which it has been produced, in such a manner that the latter put forth none but spherical gemmæ, and therefore grow, not to a long and many-branched, unicellular mycelium, but to one composed of stumpy gemmæ. On the other hand, *Mucor mucedo* treated in the same manner does not produce similar gemmæ, though, according to Brefeld (XVI.), its spores, when placed in a nutrient solution rich in citric acid, swell up to large globules from whence proceed a number of similarly

formed daughter-cells, which, however, finally perish. Numerous other fungi are also credited with the same capacity.

Now, all the *Eumycetes* capable of forming mycelia of this description can be divided into three groups. To the **first group** belong all such as, under normal conditions of nourishment, develop exclusively in the form of mycelial aggregations of gemmæ. They are therefore known as **budding fungi**. This group comprises, without exception, all the *Saccharomycetes* concerned in the fermentation industries, together with the *Mycodermæ*, *Torulæ*, &c. The **second group** consists of such *Eumycetes* as, under normal conditions, are equally well able to develop either a filamentous mycelium, or one of gemmæ. These organisms also are occasionally termed budding fungi, examples of the class being afforded by certain species of *Monilia* (Fig. 99), *Dematium*, and others. Finally, the **third group** includes all the fungi which usually produce a filamentous mycelium, the other form being only developed under abnormal conditions of nourishment. To this class belong certain species of *Mucor*, such as *Mucor racemosus*, *M. tenuis*, *M. erectus*, *M. fragilis*, *M. spinosus*, *M. ambiguus*, *M. circinelloides*, *M. alternans*, and numerous others.

However, the term "budding fungus," applied to any fungus on account of the above-mentioned behaviour, does not, in general, convey any definite information with regard to the position of that organism in the botanical system. As a matter of fact the species included in the group of budding fungi belong to very different families of the fungus kingdom.

With regard to the **anatomy and chemistry** of the *Eumycetes* cells a few general observations may be made in this place. Naked cells, *i.e.* cells destitute of membrane, occur (as motile spores) in only two—for us unimportant—sub-groups of Hyphomycetes, viz., the *Chytridiaceæ* and *Oomycetes* mentioned in subsequent paragraphs. Full information in connection with their chemical character will be found in Chapter xl. As in other cases, so also with the *Eumycetes*, the cell contents (plasma) can be divided into the nucleus and the cytoplasma with its inclusions. From the general study of cells, as treated in all botanical hand-books, and more particularly in A. ZIMMERMANN'S monograph (III.), the reader will be aware that every normal living vegetable cell contains at least one nucleus. This was first discovered by ROBERT BROWN (I.), who, in 1831, found nuclei in the cells of higher plants, whereas their presence in the cells of fungi was only ascertained by FR. SCHMITZ in 1879 (I.). It was subsequently found that the mycelium of *Hyphomycetes* is, without exception, rich in nuclei. On the other hand the mycelial cells of *Mycomycetes* contain either only a single nucleus (as in the *Saccharomycetes*), or several, as is the case, *e.g.* (according to FR. SCHMITZ (II.)) with the mycelial

FIG. 99.—Monilia candida.

Film vegetation on beer wort. Typical mycelia are present at *b* and *c*; the bud form at *a*, *e*, and *f*. Magn. about 1000. (*After E. Ch. Hansen.*)

cells of *Penicillium glaucum*, and those of the sclerotial medulla of *Claviceps purpurea*, which contain one, two, or more nuclei; and the mycelial cells of *Peziza convexula* contain several nuclei throughout. The active part played by the cell nuclei during the development of the mycelium, sporulation, and germination, has been more closely investigated by G. von Istvanffi (I.)). The reproduction of the nucleus by fission (§ 46) is mostly direct among the *Eumycetes* (*e.g. Penicillium*). Nevertheless, cases of **karyokinesis** have been observed, first by Sadebeck (II.) and afterwards by Fisch (I.). Istvanffi was unable to discern any fusion of the nucleus, as recorded by Strasburger, Zopf, and Fisch, in the formation of the oogonia of the *Oomycetes* (see § 220). It may be mentioned that the same observer also ascertained that the size of the cell nuclei in one and the same species varies in accordance with the importance attained by the corresponding parts of the thallus. Fuller particulars respecting the anatomy of nuclei, especially those of yeast cells, will be found in § 250, and on the chemistry of the subject in § 252, these details supplementing in more than one point those already given. No investigation seems to have, as yet, been conducted with regard to the presence of **centrosomes** in fungi, *i.e.* those organised constituents of cell plasma which, of late years, have become so important in the cellular physiology of animals and the higher plants.

Observations of a general character in connection with the anatomical structure of cytoplasm, and the chemical constituents of which it is composed, will also be frequently encountered in the following chapters.

Many of the Eumycetes exhibit **brilliant colouring.** Full particulars regarding the colouring matters concerned, will be found in Zopf's (X.) hand-book; and these may be supplemented by the following reference to a technical utilisation of one of these fungous pigments. To impart a red colour to rice wine, to various spirituous liquors, bread, cakes, and to the fish held in such high esteem (under the name of Macassar or red fish) in the Malay Archipelago, the Chinese employ a colouring matter extracted from a red *Hyphomyces*, which they cultivate on boiled rice. The fungus grows with vigour on this medium, and imparts thereto a red coloration; and the dried cultures, to which a preservative addition of arsenic and mustard oil is made at the time of preparation, form an article of commerce under the name Ang-Khak. C. Went has named this fungus *Monascus purpureus*, and identified it as a member of the *Thelebolea* family of *Ascomycetes* (§ 220). The red colouring matter was first examined by H. C. Prinsen Geerligs (IV.); and W. G. Boorsma (I.) afterwards found that two red dyestuffs, α-Oryzæ-Rubin and β-Oryzæ-Rubin, can be extracted from Ang-Khak, in which they are jointly present to the extent of about 1.6 per cent.

§ 220.—Fructification by Sporangia.

The separation of the thallus into two portions: the mycelium on the one hand, and the organ of fructification on the other, can be readily found in all *Eumycetes* of anything like a high stage of development; though in the lower members of the system they are only very indefinite; and may even be entirely absent, in which case the mycelium itself is converted into an organ of fructification.

The chief function of the organs of fructification is the production of structures capable of developing into new individuals of the same species as the parent organism. In the case of fungi these structures, which consequently serve for **direct propagation**, are termed **spores**; and, in accordance with the method of their production, are divided into four groups:—

(1) *Endospores*, or *Gonidia*.
(2) *Zygospores*.
(3) *Exospores*, or *Conidia*.
(4) *Chlamydospores*, or *Gemmæ*.

Setting aside, for the present, the fourth group, which occupies a position apart, it may at once be stated that, in all the more highly developed *Eumycetes*, spores of the other three groups are produced by a special organ, which branches off from the mycelium as a fruit-bearing stem: the **aerial hypha**. According to the species of the fungus and the external conditions, each of these stems may produce either one or more spores.

To take first the case of the **Endospores**: these, as the name implies, are formed within the fruit-bearing organ, or a special part thereof. Except in the very lowest species of endosporogenic *Eumycetes*, in which the mycelium itself is converted into an organ of fructification, and its contents into spores, the process takes its course along the following main lines: an upright, aerial hypha (see Fig. 91) branches off from the mycelium, and, as it proceeds to attain full growth, enlarges at the upper extremity to a usually spherical or bottle-shaped bubble: the *sporangium*, so called because in it the spores are formed. Before this happens, however, a septum is developed between the sporangium and the aerial hypha, which wall, in many cases, is not straight but curved, and projects some distance within the sporangium, as though actually an enlargement of the tip of the hypha. In some instances it assumes the form of a short pillar (Fig. 100), on which account it has received the general name *columella*. The endospores are then formed, as free cells, out of the contents of the sporangium, either the whole of these contents being drawn upon for this purpose, or only a portion as in

the case of the Mucors (§ 236). Fig. 100 represents a ripe sporangium filled with spores.

In one of the three classes of *Phycomycetes*, namely the *Oomycetes*, two kinds of sporangia, differing in structure and behaviour, are produced. The members of the one kind are termed **zoosporangia**, or **motile sporangia**, their (naked) ciliated spores issuing forth on the rupture of the sporangium, whereupon they are known as zoospores. In the other kind of sporangia there are formed—as the result of sexual fructification—large, tough-walled, quiescent spores to which the name **oospores** is given. The capacity of producing sporangia of this latter kind, which are known as **oogonia**, belongs exclusively to the *Oomycetes*, and is their distinguishing character; whereas, on the other hand, zoosporangia and zoospores are found even in the second (lowest) class of *Phycomycetes*, viz. the *Chytridiaceæ*. Zoospores occur only in these two classes, the genera of which are sub-aqueous in habit, either exclusively or during a certain period of their existence. On the other hand, the third class of *Phycomycetes*—the *Zygomycetes*—being preferably adapted to an aerial existence, is without these consequently unnecessary organs. Still less are the latter required by the more highly developed *Mycomycetes*.

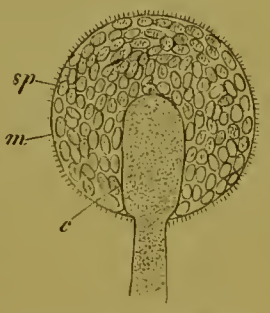


FIG. 100.—Mucor mucedo. Sporangium in optical longitudinal section. Here *m* is the sporangial membrane; *c*, the columella; *sp*, the endospores. Magn. 225. (*After Brefeld.*)

It will be found of great utility to examine how far **sporangial fructification** extends throughout the *Eumycetes*. In the members of the lower sub-kingdom, the *Phycomycetes*, the faculty of producing endogenous spores is universal. On the other hand, this faculty is present in only a single class of *Mycomycetes*, namely the *Ascomycetes*, or tube fungi, so called because the sporangium assumes a particular form, to which the name ascus (or tube) has been given. The ascus differs from the sporangium, of which it is a higher development, both in exhibiting a more definite form, and also in the number, shape, and method of formation of the contained spores (ascospores). Fuller particulars on this point will be given in § 243; since the majority of yeasts are Ascomycetes, and therefore require more thorough consideration.

The information already given renders it possible to enlarge, as follows, the scheme laid down in § 217 for the subdivision of the Eumycetes:—

			Class.
Eumycetes (Acrogenous fungi).	*Mycomycetes* (with septate mycelium).	*without* endogenous spores	All other *Mycomycetes.*
		with endogenous spores	*Ascomycetes.*
	Phycomycetes (aseptate mycelium).	*without* oogonia	*Zygomycetes.*
		without zoosporangia, but producing zygospores	
		with oogonia and zoosporangia	*Oomycetes*
		with zoosporangia	*Chytridiaceæ.*

The cause of the upward growth of the aerial hyphæ—both those bearing sporangia, and those putting forth the conidia to be described later on—has already been the subject of repeated investigation. What is it that causes these hyphæ to raise themselves from the mycelium and extend vertically upwards? In accordance with its destiny, the aerial hypha draws on the rich store of material in the mycelium, for the purpose of producing spores and filling them with an accumulation of matter. This material, being taken up in the dissolved state, needs to be freed from solvents in order to secure its deposition; and the process can only be carried out on a large scale in an environment comparatively poor in moisture, a condition that is only fulfilled *above*, but not *within*, the aqueous or damp nutrient medium. Consequently the fruit-bearing aerial hyphæ grow upwards into the air. This turning away from water is termed **negative hydrotropism**, and was first recognised, by J. Wortmann (XIV.) in 1881, in the aerial hyphæ of *Phycomyces nitens*. Communications on the same point having also been published by L. Errera (IX.), the above-mentioned state of things was afterwards recognised by G. Klebs (I.) on the basis of his researches on the conidiferous hyphæ of *Aspergillus* (*Eurotium*) *repens* and the sporangiferous hyphæ of *Sporodinia grandis*. That other functions, such as heliotropism (see § 233), may be manifested concurrently with negative hydrotropism is a matter of course. In connection with the dependence of sporangiation upon the conditions of nutrition, thoroughgoing investigations were conducted by Klebs (I.) on *Rhizopus* (*Mucor*) *stolonifer;* and this worker ascertained the definitive factor to be the percentage of moisture in the superincumbent atmosphere. J. Bachmann (II.) then showed that *Mortierella van Tieghemii* (see § 237) produces spores only when sown on a solid (not liquid) nutrient substratum, and then only provided the temperature does not fall below 20° C.

§ 221.—Fructification by Zygospores.

Zygospores are the result of the encounter of two hyphæ, or the fusion of two cells, and are produced in the following manner (Fig. 101). Two of the mycelial hyphæ coalesce, the crowns

simultaneously swelling up in the shape of a club containing a considerable accumulation of plasma. As soon as the ends of the two hyphæ come into contact (1) they flatten and an intergrowth of the membranes occurs at this spot, whereupon a septum is developed in each of the clubbed ends, which are thereby separated from their respective hyphæ. Each is thus divided into two parts : a terminal cell, to which the name **copulation cell** or **gamete** is given (2 *a*), and an interior cell, or **suspensor** (2 *b*) supporting the gamete. The partition wall between the two terminal cells is then absorbed, and the contents of these latter fuse to form a new, uniform structure : the **zygospore** or **zygota** (3 *a*). At first the outside still continues to present the appearance of two cells, but the contour soon becomes rounded off. The membrane thickens and exhibits a separation into an inner (endosporium) and an outer case (exosporium), the latter turning very dark in colour and becoming lumpy or warty on the outside. The cell contents surrounded by the endosporium contain large quantities of accumulated nutrient material.

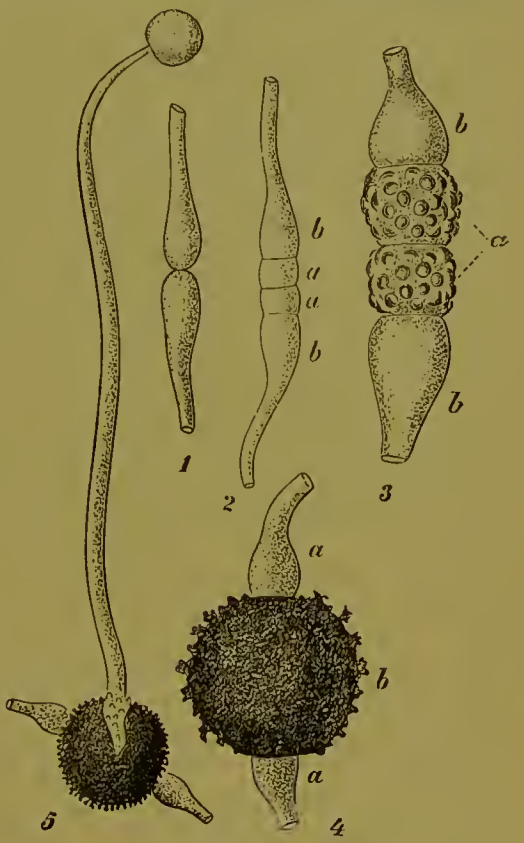


FIG. 101.—Mucor mucedo.
Formation of the Zygospores.

1. Two hyphæ in terminal contact.
2. Articulation into gamete *a* and suspensor *b*.
3. Fusion of the gametes *a* ; the membrane thickens.
4. Ripe zygospore *b* supported by the suspensors *a a*. (Magn. of 1-4, 225.)
5. Germination of the zygospore to a sporangium stem. Magn. about 60. (*After Brefeld.*)

Sooner or later the zygospore becomes detached from the suspensors, and then leads a separate existence. Being a resting cell it can, if necessary, remain quiescent for a considerable time, and then germinate on the recurrence of favourable external conditions.

The production of zygospores in the manner just described has been regarded by some mycologists as a sexual process; whilst other workers contest the admissibility of such an hypothesis.

Whichever is right, one thing is certain, namely, that a structure very similar to a zygospore can be produced without cell fusion of the kind described. In such event the resulting body is called, for the sake of distinction, an **azygospore** or **parthenospore**. This kind of spore can be formed in several ways, one of them differing from the foregoing merely in that the partition between the gametes is not absorbed, and consequently no fusion of the cell contents takes place, each gamete ripening apart to form a spore; *i.e.* two azygospores are produced. An example of this type is given in Fig. 102 (*a*). In other instances, however, the process is further simplified, inasmuch as no contact occurs between the two club-ended hyphæ, but each (or one) of the gametes (*b*, Fig. 102) develops of itself into an azygospore. In still another instance, even the endeavour of two hyphæ to approach one another is absent, the azygospores forming quite

a *b*

FIG. 102.—Formation of Azygospores in Mucor erectus.
(*After Bainier.*)

alone and independently at the extremities of branches; an example of this method of sporulation is afforded by *Mucor tenuis* (§ 235).

Ehrenberg, in 1829, was the first to observe zygospores, the organism examined being *Sporodinia grandis* (§ 235), wherein these spores attain a diameter of about 0.25 mm. It was not, however, until 1864 that the method of production and germination was made clear by the researches of A. DE BARY. Seven years later, BREFELD (I.) established the new order (class) of the *Zygomycetes*, comprising all the fungi capable of producing zygospores. These are all, without exception, *Phycomycetes*. In addition to this faculty, which distinguishes and separates them from all other fungi, the different genera of the *Zygomycetes* have, in common, various other properties which reveal their mutual relationship and therefore justify their classification into a special group, as expressed in the scheme drawn up a few pages back. This class of the *Zygomycetes* is the only one among the *Phycomycetes* that possesses any importance for the fermentation industries; it will be found treated in Section xi.

The conditions under which the formation of zygospores occurs must not be left out of consideration, since they show that, here as in other cases, the development of fungi, like that

of other living organisms, is the result of two factors; inherited properties and the sum of external active forces. The statement that zygospores are encountered solely in the class of the Zygomycetes does not imply that these fungi rely exclusively on the organs in question for their reproduction. On the contrary, this method of reproduction is rare, the ordinary and most frequent method being by sporangial or conidial fructification, as will be more specifically shown in Chapter xliii. At present we will consider the influence of external conditions on the occurrence of one or the other method of fructification. In the case of *Sporodinia grandis*, G. Klebs (II.) has shown the principal factor to be the proportion of moisture in the surrounding air. When this approaches the limit of saturation, zygospores alone are produced; but on the moisture being diminished, sporangia are formed as well; and finally, the latter are exclusively produced when the relative moisture has fallen to about 65 per cent. so that active transpiration can occur. The chemical composition of the nutrient substratum is also of influence, more particularly in that, in presence of an excess of nitrogenous substances, sporangia alone, and no zygospores, are formed, the last named requiring the presence of suitable carbohydrates for their production. It is therefore particularly interesting to observe how decisively this fungus discriminates for this purpose between individual isomeric substances. Thus zygospores are produced when the available carbohydrate consists of manite or dulcite ($C_6H_{14}O_6$), dextrose, levulose, galactose ($C_6H_{12}O_6$), saccharose, maltose ($C_{12}H_{22}O_{11}$), or dextrin; whereas sporangia alone are produced when the nutrient substratum contains one of the following carbohydrates: sorbite ($C_6H_{14}O_6$), sorbinose ($C_6H_{12}O_6$), lactose ($C_{12}H_{22}O_{11}$), raffinose, isodulcite or erythrite. It has also been found that conditions adverse to the formation of zygospores favour that of azygospores.

According to observations communicated privately by E. Ch. Hansen, the production of zygospores in *Sporodinia grandis* (as well as in a hitherto undescribed species of *Mucor*) is not so strictly dependent on the fulfilment of such conditions, but occurs readily and without any special experimental preparation. On the other hand, it cannot, so far, be induced at all in the case of several other species of *Zygomycetes*.

§ 222.—Fructification by Conidia.

The nature of this fructification and of its fundamental characteristic of differentiation from sporangial fructification lies in the circumstance that the spores, instead of being enclosed in a cell, separate by constriction from the fruit-bearing stem (conidiophore) externally, and are therefore known as **exospores**. In so far as the behaviour of the conidiophore is

concerned the process may go on according to two different types, which are diagrammatically represented in Figs. 103 and 104. Let us first consider type I. with reference to Fig. 103. The upper extremity of the conidiophore, *a*, which has sprung from the mycelium, puts forth (*b*) on its crown an enlargement, which then (*c*) becomes bounded by a septum, thus forming the first exospore. The conidiophore (*d*) then stretches a distance equal to the length of a spore, and (*e*) undergoes constriction so as to form a second spore. This operation may be repeated a second time (*f*, *g*), and even more often, thus forming a chain of spores (*h*), the topmost of which (1) is the oldest, and the lowermost (4) the youngest. The serial order of their production is arranged from the tip towards the base of the conidiophore, or the point where it branches from the mycelium; and consequently the conidia are said to be **basipetal**. The ultimate

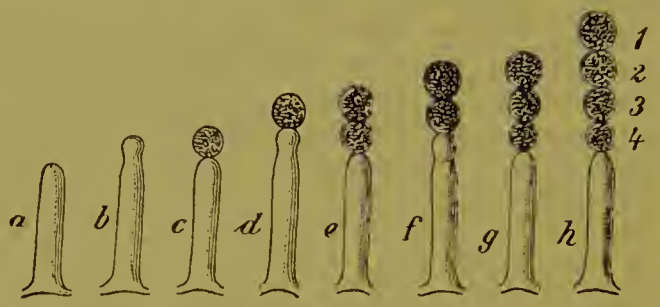


FIG. 103.—Diagrammatic sketch of the formation of conidia, according to type I. For explanation see text. (*After Zopf.*)

length of the conidiophore is the same as at the outset no matter how many spores have been formed thereon. The surmounting chain of conidia breaks away very easily; even a slight draught of air being able to bring this about, and thus distribute the spores in the form of a fine dust. Owing to their similarity to this latter the exospores have received a second appellation, and one by which they are more generally known, viz. **conidia** (from the Greek κονία = dust). An example of this type is afforded by the well-known bread mould, *Penicillium glaucum*.

In contrast to the foregoing process, wherein the conidiophore alternately increases in length and constricts to form a spore, is the second type, illustrated in Fig. 104. Here the conidiophore (*a*) ceases to grow in length as soon as the first spore (*b*) has been formed by constriction at the apex. This spore, when fully developed (*c*), puts forth a protrusion (*d*), which then grows to a second spore (*e*). This in turn continues the task of its predecessors, and consequently forms a third spore (*f*), which develops a fourth (*g*), and so on. Hence, in this type, the serial order of production of the spores (*h*) is the reverse of that in the first type, *i.e.* proceeds from the base towards the apex, and

is therefore **basifugal** or **acropetal**. In contrast to those of the first type these spores exhibit a capacity and tendency for throwing off spores laterally as well as from the apex, so that the operation, when repeated, gives rise to the formation of branched conidial clusters. An excellent example of this is furnished by *Cladosporium herbarium*, which will be described in a later chapter.

When first formed, each conidium is unicellular; and in most fungi it remains so until germination occurs. On the other hand, in some species, the interior of the spores becomes divided into compartments by the formation of one or more septa.

The production of conidia, especially those of the second type, will probably remind the reader of the process referred to as gemmation in § 219. In fact the sole morphological difference

FIG. 104.—Diagrammatic sketch of the formation of conidia, according to type II. For explanation see text. (*After Zopf.*)

between the two processes is that the one occurs on *vegetative* organs and the other on organs of *fructification*. If this difference be obliterated and the mycelium itself, at a certain period of its existence, becomes an organ of fructification, then the choice of the terms, gemmating mycelium or conidial aggregation, for the resulting cells, becomes an arbitrary one. The term, "**yeast conidium**," occurring in the older literature, must be interpreted in this sense as mostly applying to budded cells articulated from *Hyphomycetes*, and capable, from their method of formation, of being regarded as conidia, as well as resembling in their (more or less ovoid) shape the majority of the yeasts. Such forms are found in the *Dematium pullulans*, to be described in the last section but one, and also in many other fungi.

Little attention has yet been devoted to the relation between the conditions of cultivation and the production of conidia. According to G. KLEBS (I.), in the case of *Eurotium* (*Aspergillus*) *repens*, the **motive force** is probably hyphal transpiration;

and consequently a tendency to the production of spores is developed when the mycelium is constrained to exert a certain force in order to extract the water from the nutrient substratum. According to C. TANRET (I. and II.), *Aspergillus niger* will not produce conidia when obliged to develop, at 30°–40° C., in a Raulin's nutrient solution containing 0.5 gram, or more, ammonium nitrate per 100 c.c., or larger quantities of the sulphate or chloride of this base; whereas the process is stimulated by the presence of even as much as 2 grams of ammonium phosphate. Free acid, up to 0.4 gram per 100 c.c., is liberated from the first-named salts. At 20°–22° C., on the other hand, even as much as 1 gram of ammonium nitrate merely retards, without preventing, fructification. Experiments in the same direction have been made by W. SCHOSTAKOWITSCH (I.), especially with *Dematium pullulans*, as will be found in a later section relating to this organism. (See also § 229 and § 233.)

In some fungi the conidiophores, instead of remaining separate, arrange themselves in parallel order, side by side, at an early stage, and thus unite to a fascicle, to which the name **coremium** has been given. In a still higher stage of development the conidiophores—compressed together like a palisade—and the chains of conidia thereon, are enclosed in a cover; which latter is developed from the adjacent mycelium, may consist of one or more layers, and is closed on all sides when young, but afterwards opens to allow the ripened spores to escape. These forms are termed **pycnides**, an example of which is afforded by the æcidia of corn mildew, *Puccinia graminis*, which occurs in the form of red patches on barberry leaves, and, together with a number of similar parasites, constitutes the class of the *Uredinæ* or *rust fungi*. These latter, as well as the adjacent class of *Ustilagineæ* or *smut fungi*, do not come within the province of the present work, but are treated thoroughly in handbooks on plant diseases. Technical Mycology is concerned solely with certain genera of the *Zygomycetes* and *Ascomycetes*, the first named being dealt with in Chapters xliii.–xlv., and the others in succeeding chapters.

It was stated in § 220 that sporangia make their appearance in two different principal stages of development, and that the higher of the two differs from the other by greater precision as regards the number, form, and dimensions of the endospores, produced in the sporangium (or ascus). A similar distinction may also be made in connection with the formation of conidia. Among the *Eumycetes* there exists a well-defined group of fungi, in most species of which the conidiophores exhibit constancy in respect of their form and dimensions, as well as with regard to the number and form of the conidia they produce. This fact has been expressed by the bestowal of a particular nomenclature, such conidiophores being termed *basidia*, and the

group of fungi **Basidiomycetes**. It includes most of the edible fungi (*e.g.* the mushroom).

By introducing into the classification scheme laid down in § 220, the further subdivision based on the peculiarities enunciated above, we obtain the following:—

Classification of the *Eumycetes*.

			Class.
Eumycetes	*Mycomycetes* (septate mycelium)	(*a*) *not forming* endogenous spores	1. *Basidiomycetes.*
			2. *Uredineæ.*
			3. *Ustilagineæ.*
		(*b*) *forming* endogenous spores	4. *Ascomycetes.*
	Phycomycetes (aseptate mycelium)	(*a*) *forming* zygospores	5. *Zygomycetes.*
		(*b*) *forming* oospores	6. *Oomycetes.*
		(*c*) *forming neither* zygospores nor oospores	7. *Chytridiaceæ.*

The statement that the **formation of endospores** is peculiar to only a single class of *Mycomycetes*, the *Ascomycetes*, must not, however, be taken to imply that the fungi of this class are exclusively reproduced by means of ascospores. On the contrary, emphasis must be given to the fact that the production of conidia also occurs in these fungi; hence this class is richer than the other three, as regards methods of fructification.

§ 223.—The formation of Oidia and Gemmæ.

It frequently happens that the mycelium proceeds direct to the formation of conidium-like cells, without first producing conidiophores. In such cases the whole or part of the mycelium subdivides into short pieces, which are capable of subsequently germinating and thus playing the part of spores. When the mycelium in question belongs to one of the *Phycomycetes*, it must, for this purpose, produce internally as many transverse septa as will correspond with the number of resting cells to be formed. This constitutes the exceptional case referred to in § 218, where the occurrence of septa in the unicellular mycelium of a *Phycomycetes* can be observed under normal circumstances. Special partition walls are also formed in such cases in the already septated mycelium of the *Mycomycetes*. The formation of germinative resting cells by the breaking up of the mycelium, was first observed in the case of a fungus known as *Oidium lactis*, which will be more fully considered in a later section. The generic name of this fungus is now employed to distinguish the resting cells in question, which are therefore called **oidia**. Their appearance is a very ordinary phenomenon throughout the entire realm of the fungi; and it not infrequently happens that the whole of the mycelium is affected, and undergoes conversion into a coherent chain of **oidia** (Fig. 105).

In some instances this articulation and formation of germinative cells goes a step farther in order to ensure the attainment of its purpose, inasmuch as the cells are formed into veritable **resting cells**, whereas, as a rule, the oidia cannot be characterised as such, their powers of resistance being no greater than those of the mycelium from which they are elaborated. The conversion into resting cells may be effected by the thickening of the membrane of the oidia, and the absorption of nutrient material from the adjacent portions of the mycelial plasma, in consequence of which the cell in question increases in size. Owing to the large fat content of these cells, they strongly refract light, and thus show up with particular lustre

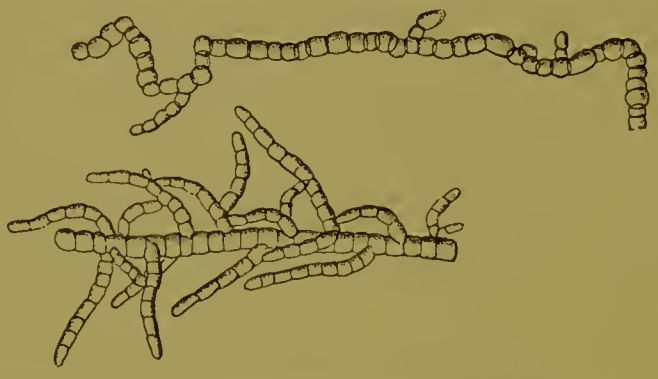


FIG. 105.—Chlamydomucor racemosus Brefeld.

Fragment of mycelium which has undergone conversion into chains of oidia. Magn. 120. (*After Brefeld.*)

(like a cut precious stone (gemma) in a necklace) against the exhausted adjoining portions of the hypha. Such a cell is termed a **gemma**, **chlamydospore**, or **brood cell**. They are more usually formed in the mycelial hyphæ, but occasionally also appear on fruit-bearing stems, which have either fulfilled their true purpose or are in the act of so doing. An example of both instances is shown in Fig. 106. In each case the gemmæ have been produced within the hypha, or conidium, and are therefore **intercalary**; though instances are known where the gemma is formed on the crown of an erect hypha, on which it is supported as by a stem, and is therefore known as a **peduncular gemma**. The first observation of the production of chlamydospores was made in 1885 by CASPARY (I.), who recognised them as a new method of fructification and gave them the name **arthrospores**, which, however, is no longer used in this sense (§ 55).

With regard to the arbitrary **production** or **suppression** of gemmæ, certain experiments were made on *Mortierella van Tieghemii* by J. BACHMANN (II.), who found that, in cultures on a solid nutrient substratum, concentration of the medium restricts

the production of sporangia and finally suppresses same, the formation of penuncular gemmæ being correspondingly increased.

The gemmæ should not be confounded with mere swellings of the mycelium, the latter being distinguishable, in the first place, by the absence of the terminal septa and definite shape, and making their appearance as a result of unfavourable conditions of existence. Lopriore found them on the germinative tubes of *Mucor mucedo*—which does not produce gemmæ at all—when the spores of this organism (placed in a suitable nutrient solution) were exposed to an atmosphere containing 60 per cent. of oxygen and 40 per cent. of carbon dioxide. Eschenhagen (I.) observed similar malformations in cultures grown in excessively rich nutrient solutions, *e.g.* a 60 per cent. sugar solution. According to M. O. Reinhardt (I.) they also occur in mixed cultures, as a result of the injurious effect, on one of the symbiotic organisms, of the metabolic products of the other.

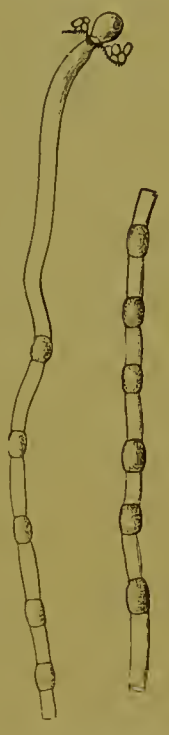


Fig. 106.—Chlamydomucor racemosus Brefeld.

On the right is a fragment of a mycelial hypha with six chlamydospores. On the left a sporangium stem with five chlamydospores; on the crown of the columella are a few endospores together with the remainder of the sporangial membrane, the bulk of which has been removed during the preparation of the specimen. Magn. 80. (*After Brefeld.*)

A fungus is said to be **monomorphous** when the same is only known to fructify in one single manner; whereas fungi exhibiting two or more methods of fructification are termed **pleomorphous**. The *Mucorineæ* (§ 235) form a good example of pleomorphism, and the same occurs in the, to us, still more interesting *Saccharomycetes*, which latter always exhibit three different methods of fructification, viz. by conidia, gemmæ, and endospores. Owing to their Protean character, these organisms occupy a peculiar position in the general morphology of the fungi; and in them we see, more than elsewhere, how the same organ can change its nature and undergo modification from one to another. According to the comprehensive researches of Hansen (XXVIII.), the yeast cell may serve (1) as a conidium, for vegetative reproduction; (2) as part of a mycelium; or (3) as an ascus and therefore producing internal spores. Finally (4), the spore is capable of not only acting vegetatively (germinating), but also, under certain conditions, of becoming an ascus by forming spores (of the second order) in its interior. Compare § 248.

Among the *Eumycetes*, the oidia and gemmæ are the most frequent varieties of fruit; but this circumstance, coupled with the simplicity and uniformity of their structure, renders these characteristics almost entirely valueless as a basis of classification for the fungi, since this classification is mainly founded on differences in the occurrence, mode of production, and development of one or more of the other three reproductive organs (endospores, conidia, zygospores), in the species to be differentiated. Fungi wherein these organs have not yet been observed, or wherein the latter appear in a form which does not permit their inclusion in the existing system, are set apart in a special class as "**Fungi imperfecti**," a term, however, expressing not incompleteness in the fungi themselves, but only in our knowledge concerning them. A few of these species, *e.g.* the so-called *Saccharomyces apiculatus*, certain *Mycodermas*, the *Moniliæ*, the *Torulæ*, &c., fall within the province of the present work, and will be dealt with fully in the final Section.

§ 224.—The Germination of Spores. Their Tenacity of Life.

The spore is ripe when it has acquired the capacity of developing into a new individual of the species from which it originated. The first stage of this development is termed **germination**, and, in the case of endospores and conidia, is a comparatively simple process. It has already been fully discussed in § 218, and illustrated in Figs. 92 and 93. Deviations from the main lines there laid down, however, sometimes occur; and with one of these we shall later on become acquainted in the case of *Saccharomyces Ludwigii*. While previous separation from the parent plant is unnecessary for the commencement of germination in the conidia, the endospores must have been set free therefrom by the decomposition or breaking down of the wall of the sporangium or ascus. More will be said on this point in § 235. Special experiments by P. Lesage (III.) have placed beyond doubt that the development to mycelium of a spore germinating on a solid nutrient substratum proceeds the more rapidly and luxuriantly the higher the water-vapour tension of the superincumbent air.

The germination of the zygospores commences by the bursting of the episporium, and the extrusion of the endosporium, in one or more places, by the pressure of the swelling cell contents. When the zygospore is submerged in a liquid, the development is of a vegetative character, a mycelium being produced; but when, on the other hand, the spore is exposed to the air, it puts forth (fructificatively) a fruit-bearing stem, which then produces spores in its turn. An example of both instances, in zygospores

of one and the same species, is given in Fig. 107. See also division 5 in Fig. 101.

The same also applies, in many instances, to chlamydospores, *i.e.* these also may germinate in two ways: either vegetatively

FIG. 107.—Sporodinia Aspergillus, Schrank.

1. Fructificatively germinating zygospore. Magn. 40. (*After Brefeld.*)
2. Vegetatively germinating zygospore. ,, ,,
3. Two ripe sporangia of this fruit-bearing stem. Magn. 150. (*After De Bary.*)
4. Azygospore. Magn. 90. (*After F. von Tavel.*)

or fructificatively. An example of a fungus possessing these properties is afforded by *Chlamydomucor racemosus*. The fructificative germination of its chlamydospores is represented in Fig. 108. The vegetative germination of these resting cells was first observed by TH. BAIL (I.) in 1857, on species of *Mucor*, this worker at the same time making the important

discovery that the chlamydospores, when submerged, develop to a budding mycelium, and not to the typical form.

In most cases the germination of the oidia is purely vegetative; it is illustrated in Fig. 109. In many fungi, certain *Mucorineæ* in particular, they are, however, also capable of fructificative germination when placed under the same conditions as the chlamydospores.

Tenacity of life, *i.e.* capacity for withstanding adverse conditions, is generally higher in spores than in the corresponding

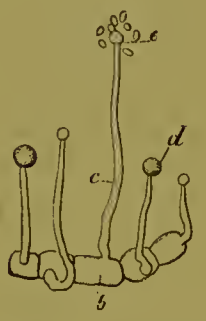


FIG. 108.—Chlamydomucor racemosus Brefeld.

Chain of five chlamydospores, each of which has put forth a sporangiophore. The sporangium of *d* is still unripe; whilst that in *c* is already destroyed, only the columella (*e*) and eight endospores being left. Magn. 120. (*After Brefeld.*)

FIG. 109.—Chlamydomucor racemosus Brefeld.

Vegetative germination of a chain of eleven oidia. Magn. 80. (*After Brefeld.*)

mycelia. Bibliographical collections on this point are given by A. DE BARY (I.), W. ZOPF (X.), W. PFEFFER (III.), and others; but only a few instances can be mentioned here. With regard to resistance towards drought and desiccation, the premier place, so far as is yet known, is occupied by the *Anixiopsis stercoraria* discovered and investigated by E. CH. HANSEN (XXVII.). The ascospores of this congener of ordinary bread mould proved still capable of germination after storage in a dry state for twenty-one years. The same observer also found life retained by the conidia of *Aspergillus glaucus* after sixteen years' quiescence, and by those of *Aspergillus flavescens* for eight years (but not longer); whilst EIDAM (III.) found the conidia of *Aspergillus fumigatus* alive after ten years; BREFELD (IV.) those of *Aspergillus flavus* after six years; C. WEHMER (IX.) those of *Aspergillus oryzæ* after more than four years, those of *Aspergillus niger* after about three years, and of *Aspergillus Wentii* after more than a year. Of course these findings do not apply to each individual spore of the species in question, the weaker specimens dying off at far earlier periods than those mentioned. With regard to the re-

sisting powers of the ascospores of *Saccharomycetes*, and the practical consequences thereof, more will have to be said in a later chapter. In the case of many fungi, the resting cells must remain quiescent for a certain period before they become capable of germinating. This applies particularly to the whole of the zygospores and oospores. On prolonged exposure to the air the contents of the spores gradually dry up, so that the membrane soon becomes wrinkled; but immersion in water causes them to swell out and resume their plumpness.

The **power of resisting heat** varies according as the heat acts on the fungus spores in the dry state (§ 76) or in presence of moisture; in the latter event they die off much more readily and speedily. Thus, it was shown by Pasteur that the conidia of *Penicillium glaucum* perish when diffused in a liquid at 100° C., whereas when moisture is excluded they will resist a temperature of 120° C. for some time, though incapable of standing 127° to 130° C. Further particulars under this head will be given in the paragraphs dealing with the moulds growing on bread, &c., in a later section.

Divergent opinions have been expressed as to the cause of the high resistance presented to dry heat by the spores of *Eumycetes*. E. Cramer (III. and IV.), on the basis of certain chemical analyses, ascribes this property to the high concentration of the cell contents, the residual water being insufficient in quantity to allow of the coagulation of the albuminoids. He found, for instance, about 61 per cent. of dry residue in the spores, but only about 12 per cent. in the corresponding mycelium; though in the former the ash content (3.1 per cent.) was smaller than in the latter (11.3 per cent.). In view of the already emphasised dependence of the chemical composition of the micro-organisms (§ 36) on the nature of the nutrient substratum, it is a somewhat risky matter to draw general conclusions from such analytical data. This also applies, be it remarked, to the results of the chemical analyses of the mycelia of *Rhizopus nigricans*, *Penicillium glaucum*, and *Aspergillus niger* (*glaucus?*) made by Marschall (I.).

In respect of **resistance to low temperatures**, what has already been stated in § 61 applies also, in the main, to the spores of *Eumycetes*. On the other hand, the mycelia, especially when rich in sap, frequently perish at a few degrees below zero C.; *e.g.* according to Molisch (V.), that of *Phycomyces nitens*. Others again, such as pressed yeast, which is poor in water, will stand very low temperatures.

As regards the resistance of the spores of *Eumycetes* towards poisons, the observations already recorded in vol. i. may be supplemented by reference to the works of O. Loew (II.), B. Joensson (I.), and F. L. Stevens (I.).

We have now learned, in connection with the general mor-

phology of the *Eumycetes*, everything necessary to enable the following chapter to be readily intelligible. In the event of any reader desiring to learn more with regard to the structure of fungi in general, the handbook compiled by W. Zopf (X.) can be consulted, after which, he may profitably study the work of A. de Bary (I.), which still—fifteen years after its first appearance—remains the standard work on mycology. The results of the labours of O. Brefeld, which in many respects were based on other fundamental views, are contained in a work published by F. von Tavel (I.).

With regard to all concerning the **general physiology** and **biology** of the *Eumycetes*, the reader can rely on the first of the three books named. During the period of rather more than a decade that has elapsed since it was completed, the subject has made considerable advances in five different directions, viz. the chemistry of the cell membrane, the requirement of mineral nutrient substances, the stimulative influence of light, chemotropism, and the production of enzymes. These questions alone will be treated in the three following chapters of the present work, since it is no part of the author's purpose to supplant existing good books. Furthermore, the reader will quickly see that there has been no stringent limitation to the subjects cited, but that every opportunity has been taken in order to gain a general view during the necessary consideration of particular points. When presented in conjunction with the treatment of questions possessing visibly practical importance, certain general explanations will be more readily appreciated by the reader than if given at the commencement of the book, where they would either pass unnoticed or fail to interest.

CHAPTER XL.

CHEMICAL COMPOSITION OF THE CELL MEMBRANE OF EUMYCETES.

§ 225.—Cellulose.

The question as to the **chemical composition** of the substance constituting the **cell membrane of fungi**, could not be agitated until some information had been gleaned in connection with the anatomical structure of these organisms. This condition was still unfulfilled at the time (1811) when H. Braconnot (II.) subjected certain edible fungi to lixiviation, and applied the name **fongine** to the residue obtained in this manner. Certainly this substance, the name of which was afterwards Latinised to **fungine**, was not a uniform body; nor does the circumstance that it was then new, justify the assumption that Braconnot was the first to prepare the cell membrane of fungi in a pure state. During the fifties, Payen subjected various fungi to lixiviation with ether, alcohol, alkalis, and acids, and made ultimate analyses of the residues. In this manner he obtained figures which fairly corresponded to the formula $C_6H_{10}O_5$. Being unaware of the immense number of isomeric substances, all possessing this elementary formula, he deduced from his data the occurrence of pure cellulose in the fungi, and denied the existence of the alleged fungine. The same conclusions were formed in succession by Schlossberger and Dœpping (I.), as also by Fromberg—whose researches were reported by Mulder (IV.)—and by A. Kaiser (I.) in 1862.

This hypothesis of the identity of the substance composing the cell membrane of fungi on the one hand, and that of higher plants on the other, was based more on prejudice than on the results of analysis, and necessarily fell to the ground on the discovery of various reagents giving peculiar and distinctive reactions with true cellulose: for instance, ammoniacal copper oxide, which was introduced by E. Schweizer (I.) in 1857, and dissolves pure cellulose without leaving any residue; zinc iodochloride, which was recommended by C. Nægeli (VII.) and H. von Mohl (I.), and gives a violet coloration; and finally iodosulphuric acid, which gives a blue coloration and was first employed by Schleiden. Soon after the introduction of the first-

named reagent, FREMY (I.) showed that, in many cases (*e.g.* mushrooms), it is incapable of dissolving the cell membrane of fungi, and, consequently that the membrane is constructed, not of pure cellulose but of a substance (of unknown constitution) to which he gave the name metacellulose. A similar opinion on this point was pronounced by A. DE BARY (III.), who gave expression to his views by employing the name **fungus-cellulose** for the substance in question. In this connection mention should also be made of Mulder's observations on the behaviour of the membrane of yeast towards iodosulphuric acid. More complete particulars with regard to this class of cell membrane will be found in § 249.

Very soon, however, doubt began to arise as to whether any special or peculiar character was really possessed by the so-called fungus cellulose. As far back as 1858 it was shown, by CARL CRAMER (I.), that the solvent action of Schweizer's reagent on true cellulose was retarded, or even entirely prevented, by the presence of foreign incrustations in the membrane. Opinion then showed a tendency to favour the idea that the membrane of fungus cells contains a fundamental substance like cellulose —and to which TSCHIRCH (III.) gave the name **mycin** —, but that this could not be detected by the aforesaid reagents owing to the presence of interspersed incrustations. This view was specially championed by K. RICHTER (I). By his investigations on a series of fungi—including *Secale cornutum*, *Agaricus campestris*, and others wherein A. DE BARY had observed the assumed fungus cellulose — this worker, in 1881, proved that when such membranes did not immediately give the reactions in question (especially with iodine), they could be induced to do so by steeping them for not less than a fortnight in a 7 to 8 per cent. solution of caustic potash. The elementary composition of the membrane so treated, he found to correspond to the formula $n(C_6H_{10}O_5)$.

Even this observation did not remain unopposed, the same hypothesis being urged against it that every investigation into the nature of the cell membrane has had to contend with ever since the days of Payen, namely, the question whether this preliminary treatment of the membrane merely results in the extraction of the extraneous admixtures, or whether it is not rather that the fungus cellulose is converted into true cellulose. However, even apart from this doubt—which will be further considered in subsequent paragraphs—Richter's observations do not disprove the assumption that a substance, differing from pure cellulose, occurs in the membrane of fungi, since, in some of his experiments, the cellulose reaction could not be observed in the preparations employed. Nevertheless, although, in view of the last-named circumstance, it cannot be admitted that, strictly speaking, Richter's experiments actually identified fungus cellu-

lose with true cellulose, it is justifiable to assume from his results that pure cellulose really does occur in the cell membrane of fungi. An attempt to support this assumption was made by I. Dreyfuss (I.) in 1893. For the purpose of extracting the cell contents and obtaining the pure membrane substance, this worker employed the method given by Hoppe-Seyler (III.). This consisted in treating the specimen with alcohol, ether, water, 2 per cent. hydrochloric acid, and 2 per cent. caustic soda, and then heating to 180° C. with concentrated caustic potash, whereby—as was then supposed—everything except pure cellulose was decomposed and dissolved. After acidifying the cooled melt the residue could then be separated by filtration, and tested for cellulose by the reagents already specified. In addition to cultures of bacteria, Dreyfuss examined certain higher fungi, viz. *Agaricus campestris*, *Polyporus officinalis*, and *Aspergillus glaucus;* and apparently found cellulose in all of them.

In view of the greater insight since afforded into the multiplicity of the isomers and analogues of cellulose, and of which the earlier workers had not the slightest idea, the successors of Richter in this branch have found it desirable to enlarge the limits of the question. Instead of the conflict respecting the identity of fungus-cellulose and true cellulose, there arose the endeavour to identify the various constituents of which the membrane is presumably constructed ; and the search for cellulose was relegated to a subsidiary position. For more comprehensive investigations in this respect we are indebted in the first place to C. van Wisselingh (I.), who, in 1898, examined no less than about a hundred species, comprising members of nearly all the orders, and most of the families, constituting the fungoid kingdom. For extracting the samples he substituted for the above method a new one devised by himself, namely, heating the fungus in glycerin, contained in a sealed tube immersed in an oil bath, and raised to 300° C. within half-an-hour. By this means the extraction is so far advanced that the residue can be tested for cellulose without opening the door to any objection. Apart from the *Myxomycetes*, with which we are not concerned, Wisselingh could only detect the presence of cellulose with certainty in two families of fungi, namely, the *Saprolegniea* and the *Peronosporeæ*, both belonging to the *Oomycetes* (*Phycomycetes*, see § 220). On the other hand, this carbohydrate could not be detected, either in the *Zygomycetes* or in any of the *Mycomycetes* examined : more particularly not in *Saccharomyces cerevisiæ*. These results agree with those obtained by E. Gilson (I.) in the examination of *Mucor vulgaris*, *Thamnidium vulgare*, and *Agaricus campestris*. The contradictions in the results of Richter and Dreyfuss will be explained in the next paragraph. Substances possibly allied to cellulose, since they have many reactions in common therewith, were observed

by Wisselingh in the fungus of the lichen known as *Usnea barbata*, and in *Geaster fornicatus* (a fungus allied to the well-known *Bovistes*), on which account he named them **usnein** or **geasterin**. On the other hand, the substance which MANGIN (I.) classed as cellulose in the case of *Mucorineæ*, *Uredineæ*, and *Ustilagineæ*, does not—according to his own reports—give the typical reactions, and hence cannot lay any claim to recognition.

It being thus certain that true cellulose has, so far, been found in only two families of fungi, it now remains to ascertain the composition of the cell membrane of the others. Thanks to the zeal of several workers, whom we shall mention shortly, satisfactory, and perhaps even surprising, information can already be given on this point; for it will undoubtedly seem strange to hear that one of the components in question is nitrogenous. This is positively known, and the substance in question will be dealt with in the following paragraphs as being most worthy our attention. Before dealing with this, however, it is advisable to state, in order to prevent misunderstanding, that the workers in question mostly employ the term fungus-cellulose in the sense of fungus membrane substance.

§ 226.—Chitin.

That the residual substance of the cell membrane, after passing through various methods of extraction, always contained nitrogen, was recognised not only by investigators—such as SCHLOSSBERGER and DŒPPING (I.)—in an age of imperfect analytical knowledge, but also more recently by W. HOFMEISTER (I.) and E. WINTERSTEIN (I.). The last-named found **nitrogen** (3.3 to 3.9 per cent.) in preparations from the cell walls of the edible boletus (*Boletus edulis*); 3.6 per cent. in the case of the mushroom (*Agaricus campestris*); 3 per cent. in the toadstool (*Cantharellus cibarius*); 2.5 per cent. in the common morel (*Morchella esculenta*); 3.3 per cent. in *Penicillium glaucum*, and 3.9 per cent. in a species of *Botrytis*. Unlike his predecessors, however, Winterstein did not rest content with the comforting belief that the extraction of the nitrogenous matter in the cell membrane had not been effected completely, but convinced himself that nitrogen was also present in such preparations as had been perfectly freed from nuclein and albumin by the previous treatment with caustic potash and Schulze's reagent. It therefore followed that the preparations in question must be partly or entirely composed of some nitrogenous substance. The same discovery, in the main, was obtained independently a few months later by EUG. GILSON (II.) in his researches on *Agaricus campestris* and ergot of rye (*Secale cornutum*). By fusing the cell preparations therefrom with caustic potash at 180° C., according to the Hoppe-Seyler method, he obtained a residue, not of cellulose but of

a substance insoluble in Schweizer's reagent, and to which he gave the name **mycosin**, the elementary composition being, moreover, ascertained to correspond with the formula $C_{14}H_{28}N_2O_{10}$. This substance is a base, the chloride of which is thrown down when concentrated hydrochloric acid is added to the aqueous solution. Mycosin itself is soluble in 2 to 3 per cent. hydrochloric acid or in very dilute acetic acid. A solution of iodine in potassium iodide, containing a trace of free acid, gives a reddish violet stain. According to WISSELINGH (I.) and E. ZANDER (I.), zinc-iodo-chloride solution varies in action in accordance with the amount of zinc chloride present, 50 per cent. producing a blue to blue-violet coloration. These last reactions closely resemble those of cellulose, and might easily be mistaken, by an inexperienced observer, as indicating the presence of that substance. This possibility will be referred to later on.

A closer indication of the position to be allocated to mycosin in the immense field of organic nitrogen compounds was afforded in a second investigation of WINTERSTEIN's (II.) by the discovery that this substance is decomposed, on hydrolysis with 3 per cent. sulphuric acid, into: *d*-glucose as the chief product; then probably other hexoses as well, and then into acetic acid and an undetermined nitrogenous organic substance. Decisive conclusion as to the method of combination of the nitrogen in this cell-membrane substance was soon afterwards furnished by WINTERSTEIN (III.) through the discovery that, when heated with concentrated hydrochloric acid, it yielded a crystallisable fission product, which proved identical with the hydrochloride of chitosamine, $C_6H_{11}O_5.NH_2.HCl$—at that time erroneously termed glucosamine, even by Winsterstein. As Winterstein at once recollected, the same behaviour is exhibited by **chitin**, *i.e.* the substance (discovered by Odier) composing the chiton and elytra of insects, spiders, crabs, and other lower animals. This substance, as was shown by G. LEDDERHOSE (I.) in 1876, furnishes under similar treatment the "**glucosamine**" discovered by that observer; and that too in the state of hydrochloride, from which latter the free base, *i.e.* **chitosamine** itself, $HO.CH_2-(CHOH)_3-CH.NH_2-CHO$, was first isolated by C. A. Lobry de Bruyn. This led to the unexpected conclusion that the cell membrane of the fungi (under examination) was to a greater or smaller extent constructed of chitin. If this should be the case, then the said preparations of the cell membrane must also exhibit the highly characteristic reaction for chitin, discovered by HOPPE-SEYLER (II.), namely, the dissociation of this substance into acetic acid and **chitosan** by exposure to fusible caustic potash at 180° C. Chitosan is basic in character, and is precipitated from its acetic solution by an excess of caustic potash. The chloride is insoluble in strong hydrochloric acid. A similar dissociation product is already known in the mycosin prepared

by Gilson in 1894; and as a matter of fact, chitosan and mycosin are identical, and can be isolated by the same method, both from animal chitin and the cell membrane of fungi. In this manner it was proved that chitin is also present in the fungoid kingdom, and is not, as was formerly supposed, of exclusively animal origin; and indeed the proof was strengthened by E. Gilson (III.) and E. Winterstein (IV.) isolating chitin, as such, from *Agaricus campestris*. The nitrogen content of this substance was determined by these workers as 6.24 per cent., a value agreeing fairly well with the 6.01 per cent. calculated from the formula ($C_{18}H_{30}N_2O_{12}$) set up for animal chitin by G. Stædeler (I.) and confirmed by T. Araki (I.) in 1895. On the basis of this formula the two hydrolytic reactions above referred to may be expressed by the equations:—

$$\underset{\text{Chitin}}{C_{18}H_{30}N_2O_{12}} + 2H_2O \overset{\text{in the potash}}{=} \underset{\text{Chitosan}}{C_{14}H_{26}N_2O_{10}} + \underset{\text{Acetic acid}}{2C_2H_4O_2}$$

$$\underset{\text{Chitosan}}{C_{14}H_{26}N_2O_{10}} + 2H_2O \overset{\text{in the conc. HCl}}{=} \underset{\text{Chitosamine}}{2C_6H_{13}NO_5} + \underset{\text{Acetic acid}}{C_2H_4O_2}$$

It may be mentioned that chitosamine, and even chitin, can be split up by the prolonged action of concentrated hydrochloric acid into ammonia and a sugar:—

$$C_6H_{11}O_5.NH_2 + H_2O = NH_3 + C_6H_{12}O_6$$

to which Berthelot has given the name **chitose**. The formation of this sugar by the action of concentrated hydrochloric acid on preparations of the cell membrane of fungi has already been observed by Stædeler (I.) without, however, the nature of the reaction having been recognised.

Bearing in mind the fact that Dreyfuss employed concentrated potash at 180° C., under which circumstances the chitin present would be converted into chitosan, the latter—according to Gilson—then behaving like cellulose in presence of certain reagents, it will be readily understood why Dreyfuss arrived at the conclusion that he had to deal with pure cellulose. According to the results obtained by Wisselingh (I.), the same conversion, and consequently the same liability to deception, also occurs when chitin is exposed for a considerable time to the action of dilute (7 to 8 per cent.) caustic potash at the ordinary temperature, *i.e.* the same treatment as employed by Richter in purifying the cell membrane preparations.

Finally, it is not inappropriate to recall that the formula given above for chitin is not yet entirely beyond dispute. It cannot at present be definitely stated whether the relative values of the bodies taking part in the foregoing reactions are accurately represented by the equations laid down by Araki; in fact we have still to face the possibility of there being a whole

series of isomeric or nearly allied substances, and therefore of the necessity of regarding the name chitin as a collective term.

The extent to which chitin is present in different groups of fungi was investigated by C. VAN WISSELINGH (I.) in researches already referred to in previous paragraphs. Its absence was confirmed by this worker in the case of *Bacteria*, *Oomycetes* (*Saprolegnieæ* and *Peronosporeæ*) and *Saccharomycetes* examined by him. In all the other species of fungi examined, however (about a hundred), the presence of chitin was invariably detected, *e.g.* in *Mucor mucedo*, *M. racemosus*, *Rhizopus nigricans*, *Penicillium glaucum*, *Tricothecium roseum*, in the sclerotia of *Botrytis cinerea* and *Claviceps purpurea*, &c. At present no instance of the simultaneous occurrence of cellulose and chitin in the cell membrane of a fungus is known. In many cases, *e.g.* the perithecial wall and the asci of *Aspergillus glaucus*, the membrane is partly composed of other substances. With the assistance of improved microchemical tests Wisselingh has also studied the local distribution of chitin in the cell membrane, and has found, for instance in the wall of the ascospores of *Aspergillus glaucus*, that chitin is present in the form of two kinds of biconvex plates.

For information respecting the amount of chitin present in certain fungi we are indebted to C. TANRET (III.), according to whose determinations—which, however, must be regarded as merely approximate estimates—about 15 per cent. of this substance is found in *Aspergillus niger*.

§ 227.—Hemicelluloses and other Carbohydrates.

Of the two main groups of carbohydrates of the formula $C_6H_{10}O_5$ entering into the composition of cell membranes, the occurrence of true cellulose in fungi has been dealt with in § 225, and there remain therefore only the representatives of the second group, namely, the **hemicelluloses** (§ 118) and other easily hydrolysed carbohydrates. Apart from the report furnished by BRACONNET (I.), we are indebted to the French chemist CHAMPIGNON (I.) for the earliest observation of these substances. From a subterranean fungus, *Pachyma Cocos Fries*, largely consumed in China under the names Fuh-ling, Fouh-ling, and Pe-fuh-ling, this worker isolated a carbohydrate of the formula $C_{20}H_{48}O_{28}$, which, on being treated with dilute acid, is converted into a liquid capable of reducing Fehling's solution. To this carbohydrate he gave the name **pachyman**. Later, E. WINTERSTEIN (II.), isolated from the edible boletus (*Boletus edulis*) a carbohydrate which is soluble in dilute acids and re-precipitated by alcohol. This substance has received the name **paradextran**, and its elementary formula is $C_6H_{10}O_5$. Closely allied thereto, and of the same composition, is **paraiso-**

dextran, which is turned blue by iodosulphuric acid and has been detected in several fungi by E. WINTERSTEIN (IV.). According to an observation made by the same worker (III.), **true hemicellulose** in the sense applied to the term by E. Schulze, also seems to occur in fungi. Two mucinous carbohydrates, known as **mycetide** and **viseosin**, but not yet closely examined, are found in various mushrooms, according to a report of Boudier's cited by A. and TH. HUSEMANN (I.). The former of these substances presumably resembles the gums, whilst the other probably is closely allied, chemically speaking, to the mucilage found in linseed and *Plantago Psyllium L.* According to an analysis made by J. L. Keller and reported by the Husemanns, **pectose** has been isolated from Fouh-ling.

Still undetermined is the chemical character of the substance giving the blue iodine reaction observed by FRIEDRICH HOFFMANN and by PAUL LINDNER (VIII.) in the case of the phlegmated hyphæ of *Dematium pullulans* and the membrane of the spores and sporogenic cells of *Schizosaccharomyces octosporus*, and also by E. CRAMER (III.) on the conidia of *Penicillium glaucum*. The substance in question is certainly not starch, but is possibly an isolichenin. Moreover, the blue reaction with iodine solution is by no means a rarity, being observed, for example, on the apices of the asci of many *Pyrenomycetes* and *Discomycetes;* whilst similar observations have been made by O. E. R. ZIMMERMANN (II.) in different species of *Mucor*. The red mould (of the genus *Fusarium*) occurring as red spots and stripes on barley and malt, where it was first observed by C. G. MATTHEWS (I.) and afterwards by CARL KLEIN (I.), excretes—through the swelling of the outer layer of the cell membrane—a mucilage which is coloured violet by iodine.

After employing the usual treatment for the determination of crude fibre, C. TANRET (I.) found, in the thallus of *Aspergillus niger*, the hard mycelium of *Claviceps purpurea*, the corpus of *Polyporus officinalis* and *Boletus edulis*, and in yeast, a carbohydrate which he named **fongose** and to which he ascribed the formula $(C_6H_{10}O_5)_6$.

The presence of a **carbohydrate of the pentosan group** $(C_5H_8O_4)$ has been detected in several species of fungi by DREYFUSS (I.). The cell membrane preparations made by WINTERSTEIN (II.) must also have contained pentosans, since they furnished 1 to 2 per cent. of furfurol when distilled with hydrochloric acid.

Basing on the results of microchemical reactions performed with various colouring matters, MANGIN (IV.) thought himself justified in assuming that **callose**—a carbohydrate allied to the pectins and discovered by him in different phanerogams—also occurs, and indeed forms the chief constituent material in the cell membrane of fungi, and of the *Ascomycetes* in particular.

At that time nothing was known regarding the presence of chitin in fungi; as, however, this latter substance behaves like the former in presence of several of the colouring matters employed by Mangin, Wisselingh (I.) thinks it not improbable that the two might be confounded.

From Winterstein's report that the fungoid cell membrane preparations examined by him did not contain more than 3.9 per cent. of nitrogen, it must be concluded that these membranes were not exclusively composed of chitin, which contains over 6 per cent. of that element, but that considerable quantities of other compounds, poorer in or altogether devoid of nitrogen, were also present.

At the present time there is very little that is reliable known with regard to the local distribution of the constituents composing the cell membranes of fungi. Not that this deficiency is due to any lack of attempts to obtain information on this point; on the contrary, these date as far back as the efforts made by W. Fueisting (I.) in 1868. At a later period the matter was energetically taken up by L. Mangin (II.), who reported that, in the *Mucorineæ* examined by him, the inner layer of the septa and aerial hyphæ consists of cellulose, whilst the outer layer is composed of pectin bodies. Unfortunately the above-mentioned observations of Wisselingh have seriously called in question the reliability of the microchemical reactions on which Mangin based his assumptions.

The reader will not expect to find here any general reports on the thickness of the cell membrane in fungi. Nevertheless, mention may be made, in this connection, of a fact determined by Fr. Eschenhagen (I.), namely, that the concentration of the nutrient solution has a direct influence on the thickness of the cell membrane of the organism grown therein.

The cell membrane of fungi also often exhibits in a high degree the capacity of swelling; which, indeed, is frequently an indispensable faculty, especially in *sporangia* and *asci*, as being the only manner in which the endospores can be set at liberty. It is, moreover, not infrequently found in the case of vegetative cells, and will be dealt with in the paragraphs on the cell membrane of yeast. Where mucination of this kind is encountered one may reasonably assume the presence of pectin substances in the cell or membrane.

According to the concordant results obtained by A. Burgerstein (I.), M. Niggl (I.), and C. O. Harz (II.), lignification of the membrane does not seem to occur in *Mucor mucedo*, *Penicillium glaucum*, *Aspergillus glaucus*, and *Saccharomyces cerevisiæ*, or, according to the observations of the last-named worker, in *Mucor nigricans*, *Aspergillus conoideus Spreng.*, *Asp. candidus Link.*, *Asp. flavescens Rob.*, *Cephalothecium roseum*, *Tuber cibarium*, *T. æstivum*, *Claviceps purpurea*, and *Torula*. On the other

hand, the lignin reaction (with sulphuric aniline sulphate) was furnished by the large pileated fungi, though whether the presence of lignin is thereby proved must remain an open question.

Not infrequently a deposition of colouring matter is found in the cell membrane, usually in such a condition as to be incapable of extraction by any known solvent; this is the case with the conidia of *Aspergillus* and *Penicillium*. In other coloured fungi the colouring matter is embedded in the plasma; of this a fine and technically important example has already been given in § 219.

Whether the waterproof character of some cell membranes, *e.g.* in the conidia of *Penicillium* and *Aspergillus*, should be attributed to the deposition of **excreted fatty or waxy substances**, must be left undetermined. Biologically this phenomenon is important since it prevents the penetration of toxic substances from the surrounding aqueous medium, and thereby also opposes the attempts of the mycologist to kill such fungi by means of aqueous toxic solutions.

Deposits and incrustations of **calcium oxalate crystals** are of very frequent occurrence in the membrane of fungi, especially on the surface of the spores. In many cases their presence and appearance afford characteristic indications valuable for the purposes of classification.

CHAPTER XLI.

MINERAL NUTRIENT MATERIALS.

§ 228.—Alkalis.

A GLANCE through the existing analytical data concerning the **ash constituents of fungi** (see for example the handbook by J. KŒNIG (I.)) will soon reveal that the chief of these constituents are **phosphoric acid** and **potash.** The latter seldom forms less than one quarter, and is generally about one half of the total weight of ash, sometimes even rather more; the ash of truffles, for instance, according to an analysis by LIEBIG (II.), contains 54.5 per cent. of K_2O and 33.0 per cent. of P_2O_5. From this circumstance alone it may be concluded that, as has already been placed beyond doubt in the case of green plants, **potassium** is also of importance to fungi. The first experiment made in order to clear up this question was instituted by NÆGELI (IV.), and the results obtained led this worker to assume that potassium is so far non-essential to the growth of fungi that it can be replaced by **rubidium** or **cæsium**; but that one or other of these three must invariably be present.

With regard to cæsium, all subsequent investigators, however, agree that this metal is unsuitable for replacing potassium for the purpose in question. Instances of this are furnished by S. WINOGRADSKY (XI.) in his culture experiments with *Mycoderma vini;* W. BENECKE (II.) in the case of *Penicillium glaucum* and *Aspergillus niger;* and subsequently by E. GUENTHER (I.) for *Mucor corymbifer*, *Rhizopus nigricans*, and *Botrytis cinerea.*

Opinions are divided as to the suitability of rubidium for replacing potassium. An affirmative result was furnished by O. LOEW's (VII.) culture experiments with a species of *Penicillium*, and by Winogradsky with the film-fungi already mentioned. On the other hand, in the experiments of W. BENECKE (II.) potassium was found replaceable by this allied metal, but only in cases where merely vegetative development was in question. Finally, the experiments of E. GUENTHER (I.) furnished no uniform results: the cultivation in solutions containing rubidium, but no potassium, being successful in the case of *Botrytis cinerea*, but not so with *Rhizopus nigricans.* Now, in order to rightly appreciate these results it will be necessary to bear in mind the great difficulty experienced in completely freeing the

rubidium salt from the accompanying potassium salts. This difficulty is, moreover, accentuated by the comparatively ready solubility (even at simple boiling temperature) of the glass of the culture vessels, the consequence being that a little alkali finds its way into the nutrient solution from the glass during the sterilising process. In order to eliminate this source of error several workers have already recommended the use of metallic culture vessels for the purpose in question, but soon had to abandon same on account of the toxic action and consequent retardation of development produced on the sowing. This was observed, in the case of silver, by RAULIN (III.), whose similar observation in the case of tin was confirmed by W. BENECKE (III.). This last-named worker also excluded aluminium from the list of suitable metals, for the reason that it sustained corrosion and therefore caused an alteration of the nutrient solution, although TH. BOKORNY (I.) employed it, apparently with good results, in his experiments on the nutrition of *algæ*. Finally platinum, which, according to Bokorny, has a poisonous effect on the green thallophytes in question, was found by BENECKE to be innocuous in the case of *Aspergillus niger*; it is, however, too expensive to use for large series of experiments. The difficulties encountered in purifying the nutrient salts, in order that the experiments conducted therewith may be perfectly reliable, can best be appreciated by the aid of the following data, for which we are indebted to W. BENECKE (II.) and E. GUENTHER (I.). The figures relate to the minimum quantity of KCl which will enable the development of the sowing to proceed when added to 100 c.c. of a nutrient solution previously free from potash. *Aspergillus niger* is sensitive to 0.02 mg., *Rhizopus nigricans* to 0.01 mg., *Mucor corymbifer* to 0.02 mg., and *Botrytis cinerea* to 0.01 mg. of KCl. The essential requirements of the fungi in respect of potash are therefore very moderate; and, in fact, if the necessary quantity be exceeded, to the extent of several units per cent., the growth may be injured. The maximum amount of potash salts which *Rhizopus nigricans* will stand, and still continue not only to grow but also to fructify, has been determined by E. GUENTHER (I.) as follows: KCl, about 7.5 per cent.; KNO_3, about 7 per cent.; whilst in the case of K_2SO_4 the organism will still bear up to 10 per cent. (concentrated solution) very well.

In opposition to the concordant results obtained by earlier workers, CARL WEHMER (IV.) assumed that sodium is able to replace potassium as a nutrient material for fungi. This view was, however, disproved by the experiments of W. BENECKE (III.) on *Aspergillus niger*, an undescribed species of *Penicillium*, *Mucor stolonifer*, *Botrytis cinerea*, and a pure-culture wine yeast from Winningen; these results being also strengthened by E. GUENTHER'S (I.) culture experiments with *Mucor corymbifer*,

Rhizopus nigricans, and *Botrytis cinerea*. The rule therefore still holds good that sodium is of no appreciable utility as regards the nutrition of fungi, and can be entirely dispensed with. The maximum quantities of the salts of this metal that can be present in the nutrient solution without injury, have been determined by E. Guenther, in the case of *Rhizopus nigricans*, as follows: NaCl, 12 per cent.; $Na_2SO_4 + 10$ aq., 26 per cent.; and $NaNO_3$, 6 per cent.

With regard to **lithium**, W. Benecke (III.) has shown—in refutation of the contrary assumption by Naegeli—that this metal is not a foodstuff for fungi, although a strong stimulant. When lithium salts were present in the nutrient solution it was found that the conidia of *Aspergillus niger* did not germinate, and that no conidia were formed in the case of an unspecified species of *Penicillium*. The extent to which the various species are sensitive to the action of this metal must fluctuate considerably, since, whilst E. Guenther (I.) found 0.05 per cent. to be the largest addition of lithium nitrate that *Rhizopus nigricans* could stand and still continue to thrive, H. M. Richards (I.) was able to observe that *Aspergillus niger* gave a crop of double the usual size when the nutrient solution of saccharose and mineral salts was treated with an addition of 0.3 to 0.5 per cent. of lithium chloride.

§ 229.—Metals of the Alkaline Earths.

According to Nægeli (IV.) **magnesium** is non-essential for the development of fungi when the latter have at disposal one of the three alkaline earths: **strontium**, **barium**, or **calcium**. The assumption that fungi can grow without magnesium has, however, been disproved by Winogradsky (XI.), who showed that the latter is indispensable for the development of *Mycoderma vini*. The same results were obtained by Adolf Mayer in culture experiments with beer yeast; by H. Molisch (II.) and W. Benecke (I.) with *Penicillium glaucum* and *Aspergillus niger*; and by E. Guenther (I.) with *Mucor corymbifer*, *Rhizopus nigricans*, and *Botrytis cinerea*. How sensitive and responsive the fungi are to a small addition of magnesium is evident from the observation recorded by Benecke (III.) as to the considerable difference in development exhibited by two, otherwise equal, specimens, the one grown without magnesium and the other in a medium containing 0.0025 mg. of crystalline magnesium sulphate per 25 c.c. Similarly, E. Guenther (I.) ascertained, in the case of magnesium sulphate, ($MgSO_4 + 7$ aq.), that a minimum addition of 0.005 mg. was necessary to induce a sowing of *Rhizopus nigricans* to grow at all.

In refutation of an earlier assumption by Sestini, it has been shown by H. Molisch (II.) and W. Benecke (I.), and after-

wards confirmed by E. Guenther (I.), that not only calcium, barium, and strontium, but also the closely allied metals **beryllium**, **zinc**, and **cadmium** are unsuitable for replacing magnesium; and that, in fact, they behave as poisons when added in slightly larger amount to the nutrient medium. An addition of 0.02 *per mil* of cadmium sulphate or cadmium chloride is sufficient for *Aspergillus* and *Penicillium;* whilst for *Rhizopus nigricans* 0.001 *per mil* is enough. According to Guenther, an addition of 0.2 per cent. of beryllium chloride is necessary to restrict the development of the last-named *Phycomyces.*

With regard to zinc, it was observed by J. Raulin (I. and III.) before 1870, in his experiments with *Aspergillus niger*, that the mycelial development of this fungus could be considerably facilitated by a small addition of zinc sulphate to the nutrient medium. The conclusion drawn therefrom that, in contradistinction to the earlier discoveries of the same worker, zinc is indispensable to the structure of the fungus in question was, however, unable to stand subsequent investigation. Both in this fungus and in the case of *Penicillium glaucum* and *Botrytis cinerea* it was found by W. Pfeffer (II.), H. M. Richards (I.), and Ono (I.), that the action of zinc is stimulative (§ 81) in the sense of H. Schulz's law (I.). Even an addition of 0.0005 per cent. of zinc sulphate to a nutrient solution of, *e.g.* saccharose and mineral salts, resulted in a considerable increase in cropping. This attained double the yield (furnished in the absence of zinc) when the addition reached the optimum amount of about 0.003 per cent. of zinc sulphate; but, on raising the addition to 0.05 per cent., a poisonous action was observed. *Rhizopus nigricans* seems to be still more sensitive, since, according to E. Guenther (I.), it will not stand more than 0.01 gram of zinc sulphate in 100 c.c. of nutrient solution. A noteworthy observation made by several workers is that this stimulation is really a kind of fattening process, the stimulative influence being confined to the development of the mycelium, that is, to the vegetative portion of the thallus; whilst the production of conidia, or organs of fructification, is retarded, and even entirely suppressed. Supported by other experimental results (relating to copper as well as zinc), Andr. Richter (I.) has pointed out that, in such a state of dilution, the salts (*e.g.* zinc sulphate) are no longer capable of acting as such, but—in accordance with the theory of dissociation—are more or less separated into their components, the ions Zn and SO_4, which are therefore the real stimulants. Moreover, because the extent of the dissociation is also dependent upon the nature of the solvent—in this case the nutrient solution—the action exerted by an addition of such saline stimulants is also determined thereby. The present is a suitable occasion for referring to the labours of Th. Paul and B. Krœnig (I. and II.), Scheurlen and Spiro (I.), and others, to whom we are indebted

—since the publication of vol. i.—for the application of the dissociation theory to the study of the action of poisons on micro-organisms (§§ 79 and 81), and for the useful conclusions that may be drawn therefrom with regard to practical disinfection.

In connection with magnesium, indirect reference has already been made to calcium, inasmuch as Naegeli's assumption that this metal could replace magnesium was stated to have been refuted. There still remains, however, the less important question whether calcium is at all essential to the structure of fungi. On this point Winogradsky, in a previously cited research, found *Mycoderma vini* still capable of thriving in a nutrient medium free from calcium (and also from barium and strontium). The same results were obtained by H. Molisch (II.) and W. Benecke (II.) in their experiments with *Aspergillus niger*: so we may well assume that calcium is not essential to the growth of fungi. Until a short time ago it was considered that this peculiarity afforded a thorough means of distinction between the fungi and green plants. However, it has recently been found by H. Molisch (IV.) that certain (but not all!) of the algæ will thrive in the absence of calcium, *e.g. Stichococcus bacillaris* Nægeli, *Ulothrix subtilis* Kuetzing, but not *Vaucheria* or *Spirogyra*. For the future, therefore, the axiom must be changed, and calcium regarded as indispensable for the higher green plants, but not essential to the fungi and to certain algæ.

So far as barium and strontium are concerned, it has been placed beyond doubt that these two metals are not only useless, but also injurious, and act as poisons toward the fungi. Thus, for example, in the experiments of E. Guenther, the development of sowings of *Rhizopus nigricans* ceased in presence of 1.0 per cent. of barium nitrate, or 1.5 per cent. of strontium nitrate, in the nutrient solution. Even calcium, it may be remarked in passing, is capable of acting injuriously when present in larger quantities, the last-named worker having found the limit of safety to be 4 per cent. of calcium nitrate in the case of the same *Phycomyces*.

§ 230.—Elements of the Iron Group.

On the fact that **iron** had been shown indispensable to the formation of chlorophyll, and therefore essential to the assimilative power of green plants, was based the opinion, long unanimously current among mycologists—*e.g.* Adolf Mayer (I.) in connection with yeast, and C. Nægeli—that the fungi, being destitute of chlorophyll, do not require iron. This assumption was first put to the test by J. Raulin (I.) in 1870, the results showing that, in presence of iron, cultures of *Aspergillus niger* throve more vigorously in solutions of nutrient salts containing

iron, the weight of the crop being about double that obtained in cultures without iron. Presuming the experiments to have been properly conducted, this result would justify the assumption that iron, though very beneficial to the growth of the fungus in question, is not indispensable. Raulin, however, suspected that his "iron-free" cultures did not deserve their name, but contained a small proportion of this metal as an inseparable impurity in the other salts. On these grounds, though without proof, Raulin concluded that iron is indispensable for the development of *Aspergillus niger*. The contrary opinion, viz. that iron is non-essential to fungi, was expressed by Cugini (I.) in connection with a pileated fungus, and by A. Schulz (I.) for *Mycoderma cerevisiæ;* though, as neither of them worked with solutions that could be guaranteed free from iron, the question still remained undecided. Hans Molisch (I. and II.) attempted to settle it, without, however, succeeding in producing a perfectly iron-free culture. Nevertheless, his results tend to prove the indispensable character of the element in question, inasmuch as, in cultures as free as possible from iron, the spores of *Aspergillus niger* did not develop beyond the formation of a sickly mycelium, whereas the specimens treated with an addition of iron not only exhibited luxuriant growth, but also produced an abundance of spores. Similar results were obtained with sowings of pressed yeast cells, spores of *Mucor racemosus*, and a species of *Penicillium*. Bearing in mind the observation (communicated by Molisch) that a ferruginous ash is furnished even by cultures grown in a medium from which iron has been, as far as possible, eliminated, and therefore that even imponderable amounts of iron are greedily absorbed, one is constrained to share the conclusion formed by Molisch, that iron is very probably indispensable to the development of fungi. The objection urged by C. Wehmer is based on observations that cannot be regarded as perfectly reliable. The hypothesis was raised by H. Molisch (I.), and shortly afterwards by A. B. Macallum (II.), that iron occurs, in plants generally and in fungi particularly, in the form of organic compounds, and is therefore undetectible by the ordinary reactions. Macallum (I.) then explained that the chromatin in the nuclear structure (§ 252) of the cell is the chief seat of these organic compounds of iron. The instances cited in support of this view, however, have been strongly criticised, since they were based on experimental methods the unreliability of which has been demonstrated by Arthur Meyer, Carl Mueller (I.), and G. Gilson (I.), and also partly admitted by Molisch (III.) himself. Nevertheless, as may be concluded from later observations, the assumption itself seems appropriate. The first of these observations was made in 1877 by Lubavin, who detected the occurrence of iron in the molecule of the paranuclein prepared from milk casein. Further reports in this connection were made by A.

Ascoli (I.), who succeeded in ascertaining the important fact that the plasmic acid (§ 252) isolated from the nuclein of yeast contains about 1 per cent. of (masked) iron, which is probably attached direct to the phosphorus atom. Attempts have already been made to utilise this fact in pharmacy and medicine; and since 1900 there has been recovered from yeast grown on a highly ferruginous nutrient medium, a fission product of nuclein, to which the name **ferratogen** has been given, and which contains about 1 per cent. of iron in organic combination and readily absorbed in the intestines. According to G. Marpmann (II.), the iron in fungi (and especially in *Penicillium*) is usually in the ferrous state, and only exceptionally present in a higher stage of oxidation; this has been demonstrated by treatment with hydrochloric solutions of potassium ferro- and ferri-cyanide. An exception is afforded—at least according to R. Kusserow (II.)—by yeast (pressed yeast in particular), the stability of which is said to be influenced by its abundant content of ferric phosphate. In addition to its part as an indispensable foodstuff, iron also seems to act as a stimulant: according to the indications afforded by comparative experiments undertaken by H. M. Richards (I.).

The metals allied to iron, namely **nickel**, **cobalt**, and **manganese**, have been tested on *Aspergillus niger* by Molisch (II.) with regard to their capability of replacing the first-named, but were found unsuitable. This does not, however, imply that the fungi entirely reject these elements. On the contrary, it has been shown by W. Pfeffer (II.) and H. M. Richards (I.) in cultures of *Aspergillus niger* and *Penicillium glaucum*, that cobalt and nickel, in the condition of sulphates, can exert a similar stimulative action to that already ascribed to zinc (§ 229). The optimum quantity of the salt of the first-named metal was found to be 0.002 per cent., which gave a crop almost double that from the check experiment without cobalt. In the case of nickel sulphate the corresponding quantity is 0.033 per cent. (consequently much greater), and this increases the crop two and a half to three fold.

As long ago as 1884, **manganese** was detected by J. Schlossberger and O. Dœpping (I.) in various mushrooms—*e.g. Agaricus deliciosus*, *Ag. arvensis*, and *Amanita muscaria*—and subsequently by Bissinger (I.) and others in *Lactarius piperatus*. Both physically and chemically this element seems to play a very noteworthy part. From what has already been stated in § 215 of Vol. i., the reader is aware that many fungi contain oxidising enzymes allied to the laccase in the sap of the lac tree. Now, in the ash of the latter, Gabriel Bertrand (III.) has discovered up to 2.5 per cent. of manganese. Subsequent experiments have led this worker to conclude—though this has not yet been confirmed—that manganese is the real active agent in the

oxidases, by reason of the convertibility of its protoxide, this being readily oxidised to peroxide, which in turn as readily parts with oxygen and is reduced to the protoxide. Consequently, in BERTRAND'S (VI.) opinion, the organic constituents of the oxidases merely play the part of carriers of manganese. For the purpose in view, this latter cannot be replaced by any allied or other metal.

According to the results obtained by RICHARDS (I.) in culture experiments with *Aspergillus niger*, **aluminium** is not only non-essential as a foodstuff, but has no appreciable action as a stimulant.

§ 231.—Sulphur, Selenium, Silicon, Phosphorus, Arsenic.

Strictly speaking, it has not yet been proved that **sulphur** is essential to the growth of fungi; that it is so having been concluded from the (still disputed) assumption that this element forms an important constituent of the albuminoids. The attempts hitherto made to carry out perfectly convincing experiments, in nutrient media, positively free from sulphur, have proved futile. Thus, neither ADOLF MAYER nor E. GUENTHER (I.) succeeded in fully eliminating this element from the saccharose used in the preparation of nutrient solutions, a few thousandths of a per cent. remaining in combination as an ineradicable impurity. According to the investigations of Nægeli, sulphates as well as sulphites and hyposulphites may serve as a source of sulphur; but ammonium thiocyanate and sulphurea are unsuitable. A careful confirmation of this report is the more desirable because Adolf Mayer, in his cultures of beer yeast, found sulphates unsuitable for this purpose.

Selenium appears to be incapable of replacing its near ally, sulphur, as a nutrient material for fungi. At any rate, the experiments of E. GUENTHER (I.) with *Rhizopus nigricans* have shown that an addition of even 0.0005 per cent. of sodium selenate will suffice to prevent the germination of spores of this fungus in a nutrient solution of glycerin and mineral salts.

Silicon also, according to J. RAULIN (III.), must be included in the list of foodstuffs essential to fungi, though no support to this view is afforded by the later cultivation experiments conducted on this point by H. M. RICHARDS (I.). Nevertheless—in view of the observation (unfortunately not followed up) of E. WINTERSTEIN (I.) that the ash of his so-called fungocellulose (§ 225) consisted almost exclusively of silica—it may be regarded as not impossible that silica (as is undoubtedly the case in the higher plants), while not *essential* to the structure of fungi is very useful for strengthening their membranes. The present

is a suitable opportunity for mentioning a nutrient solution (*Liquide Raulin*) still used in French laboratories, for both moulds and fission fungi. It was compounded by RAULIN (I. and III.) on the basis of his observations, which we now know to have been somewhat imperfect, and is composed of :—

	Grams.		Grams.
Water	1500·0	Potassium carbonate .	0·60
Saccharose . . .	70·0	Tartaric acid . .	4·00
Ammonium nitrate .	4·0	Ferric sulphate, zinc sulphate, potassium sulphate, each . .	0·07
Ammonium phosphate .	0·6		
Ammonium sulphate .	0·4		
Magnesium carbonate .	0·40		

Next to potash, **phosphoric acid** is the most important ash constituent in fungi. Existing analyses place the figures at between 15 and 60 per cent.; though it should be mentioned that not all the reports on this point are of equal value, some of them relating to cases where insufficient regard was paid to the volatility of phosphoric acid in presence of carbon, during the preparation of the ash. The consensus of existing experiments favours the assumption that phosphorus is essential to the structure of fungi; and, moreover, it is known that this element forms an important constituent of the nucleins (§ 252). The eagerness with which this foodstuff is absorbed from the medium can be gathered from an instance recorded by J. SCHLOSSBERGER and O. DŒPPING (I.), wherein a pileated fungus, *Dædalea quercina*—of the *Polyporea* group, and allied to the dry-rot fungus—was found to have completely extracted the phosphoric acid from decayed oak wood. With a view to ascertaining the local distribution of phosphoric acid among the individual constituents of the cell contents, L. LILIENFELD and A. MONTI (I.) have described a process, which consists in first immersing the preparation in a nitric solution of ammonium molybdate, whereby a yellow precipitate of ammonium phosphomolybdate is produced in such parts of the cell where phosphoric acid, capable of reacting, is present. Then, after washing out the preparation, it is exposed to the action of a reducing agent (such as a 20 per cent. solution of pyrogallol); this reduces the molybdic acid to lower stages of oxidation, which, by their brown or blue colour, reveal the locality and distribution of the phosphoric acid in the cell. In this manner the hyphæ of *Botrytis cinerea*, the spores of the same fungus, and the cells of *Mucor* and *Saccharomycetes*, have been tested for phosphorus by S. POLLACCI (I.). According to its authors, the method is capable of detecting not merely the phosphoric acid in combination as inorganic orthophosphates, but also, in many cases, that present in organic combination, especially in the nucleins. It has, however, been found by L. HEINE (I. and II.) that the same colour reaction

is furnished by various substances devoid of phosphorus—many albuminoids in particular—so that it is impossible to obtain by this method any reliable differentiation, especially between the nuclein-like and the albuminoid constituents of the cell.

The question how far phosphorus can be replaced as a foodstuff by its ally, **arsenic**, after having been answered in the negative by J. Stoklasa for the higher phanerogams, and by H. Molisch (IV.) for different algæ, was examined by E. Guenther (I.) in connection with *Rhizopus nigricans*, the same result being obtained. This notwithstanding, compounds of arsenic are attacked and assimilated by many fungi, provided they are present in insufficient quantity to exert any poisonous action. B. Gosio (II.) was the first to show that *Penicillium glaucum*, *Aspergillus glaucus*, *Asp. virens*, *Mucor mucedo*, *M. racemosus*, *Cephalothecium roseum*, and others, in suitable nutrient media will convert arsenious acid into volatile compounds having an odour of garlic, and with such a degree of certainty that an addition of 0.02 m.g. of Na_2AsO_3 can be detected in 10 c.c. of milk by this means. This new fact is of practical interest to official chemists and hygienists, in connection with the toxic phenomena produced by wall papers, &c., containing arsenic. The chief literature on this subject has been collected by R. Abel and P. Buttenberg (I.). Attempts have been made to explain these phenomena by assuming that the printed arsenical pigments come off in the form of dust; and this view was recently accepted by O. Emmerling (I.), since neither he, nor G. Marpmann (V.) since, have succeeded in liberating arseniuretted hydrogen from cultures of the aforesaid fungi (or of *Mucor corymbifer* and *Aspergillus fumigatus*), on arseniferous nutrient media. On the other hand, the probability that volatile organic compounds of arsenic are formed has been shown by Gosio (IV.); and one such has been made known by P. Biginelli (I.) as dimethylarsine, $As(C_2H_5)_2H$, which is a near ally of the extremely poisonous substance cacodyl and emits an odour of garlic. The advantage accruing to the analyst from Gosio's observation is the possibility of replacing the Marsh test for arsenic by a shorter, microbiological method, wherein the substance under examination can be tested without the necessity for a tedious preparation (destructive of the organic matter!). The method as recommended by Gosio is performed in the following manner: A cut is made in a clean piece of raw potato, and after inserting therein a little of the substance (*e.g.* aniline dyestuff) to be tested for arsenic, the potato is placed in a Roux test glass (potato glass) and sterilised for fifteen minutes under a pressure of one atmosphere. Abel and Buttenberg (I.) recommend the employment of a pap made from the crumb of white or brown bread and contained in an

Erlenmeyer flask; and this has been found useful in the present author's laboratory. When cooled, the substratum is inoculated with an organism known to be suitable for the purpose in view, and the whole is then left to stand for a day at about 37° C. If the substance contain arsenic, the contents of the tube or flask will, at the expiration of that period, emit a strong and persistent odour of garlic. Gosio cites, as the most energetic assistant in this connection, the (presumably new) *Penicillium brevicaule* discovered by him on arsenical wall paper. In order to be sure in the first experiment, the liberated vapours may be passed into an oxidising liquid, *e.g.* permanganate solution, and examined by the Marsh test. By the aid of this microbiological method Fr. Abba (I.) succeeded, in a case under judicial investigation, in proving the presence of arsenic in a sample of Indian meal; then in the urine of a patient treated with arsenic; and also in illuminating gas from the Turin mains, the arsenical constituents being collected by passing the gas through caustic potash. In the same manner, several hundred samples of pelts were tested for arsenic (§ 157) by Gosio (V.), and also later on by Abba (II.). In proof of the delicacy of the method, the last-named worker states that, whilst, with the Marsh apparatus, no arsenic could be detected in a piece of hide measuring 5 sq. c.m., an unequivocally affirmative answer was obtained by the biological method from a piece one-fiftieth the size, *i.e.* only 10 sq. m.m. This method has also been employed, and its delicacy appreciated, by G. Mopurgo and Alb. Brunner (I.) for the examination of colouring matters used in the provision industry. Samples containing not more than 0.2 m.g. of arsenious acid per 10 grams gave merely a very slight, doubtful mirror in the Marsh apparatus; but when treated in the manner prescribed by Gosio they disengaged a strong odour of garlic within a few hours. A still more favourable opinion is expressed by W. Scholz (I.), R. Abel and P. Buttenberg (I.), Br. Galli-Valerio and C. Strzyzowski (I.), who found the limits of delicacy of the process correspond to 0.02 to 0.05 m.g., and 0.001 to 0.01 m.g. of arsenic respectively. The reaction was not given by other metallic poisons, such as compounds of antimony, lead, and bismuth in particular. Out of more than forty different species of mould fungi that have up to the present been examined with regard to their suitability for this method, *Penicillium brevicaule* has proved *facile princeps*. On the other hand, *Aspergillus flavus*—which, according to R. Schmidt (I.), has a very powerful reducing action—*A. niger*, *A. subfuscus*, *A. fumigatus*, *Penicillium glaucum*, *Mucor mucedo*, and others, have been found unsuitable, the odour of garlic being either entirely absent, or else masked by the fusty smell of the mould fungi.

In view of the details given in the foregoing, it may be

stated that, according to the present state of our knowledge, nine elements are indispensable for the structure and complete development of the Eumycetes, namely—

Carbon, hydrogen, oxygen,
Nitrogen, sulphur, phosphorus,
Potassium (or rubidium), magnesium, and iron.

CHAPTER XLII.

STIMULATIVE INFLUENCES—GENERAL REMARKS ON THE ENZYMES OF EUMYCETES.

§ 232.—Influence of Light on the Development of the Eumycetes.

According to an old colloquial expression, the fungi are children of darkness. This statement, however, is only appropriate in certain instances, more particularly in the subterranean species, *e.g.* the truffle, and is by no means capable of general application. The first to protest against this generalisation was E. M. Fries who showed, in 1825, that light is indispensable to the normal development of certain fungi. Following his lead, a large number of workers have since investigated the connection between light and fungoid life; but only a few of the results obtained can now be briefly recapitulated, namely, those concerned with the fungi of interest to readers of the present work. We will also leave out of consideration the older reports dealing with the malformed growths—due to defective illumination—of fungi in mines, where they drag out a miserable existence; and also the (barren) mycelial masses found in mine shafts and headings, and described, in the older literature, under the collective name of *Byssus*. Information on this question will be found in a work by Elfving (I.), to which we shall have occasion to refer later on.

The first phenomenon we shall now consider is one that will become apparent on even merely superficial observation, namely **phototropism**, or the influence exerted by light on the direction of growth. At the outset research was confined to the narrower field of the form of illumination most common under natural conditions, viz. by the sun's rays (**heliotropism**); and, according as this influence proved stimulative, retardative, or inert, the fungi affected thereby were classed as positively heliotropic, negatively heliotropic, or aheliotropic. An example of each of the two latter possibilities was furnished by J. Schmitz (I.) in 1843 and by Kraus (I.) in 1876, the latter of whom found *Rhizopus nigricans* (*Mucor stolonifer*) presumably aheliotropic. Schmitz observed that the mycelial threads at that time classed

independently as *Rhizomorpha*, but subsequently assigned to the cycle of development of *Agaricus melleus*, turned away from the light, though Brefeld (III.) was unable to confirm this behaviour. E. Chr. Hansen (XXII.) made us acquainted, in 1897, with three new examples of negative heliotropism, in species of the families *Coprinus* and *Agaricus*. With regard to all the remaining fungi examined for their sensitiveness to light and found to be exclusively positive in their heliotropism—such, for example, as the conidiophores of *Peziza Fuckeliana* examined by G. Winter (I.), the sporangial hyphæ of *Mucor mucedo*, *Phycomyces nitens*, and a species of *Pilobolus*, examined by Kraus (I.) and Vines (I.), and the stalk of *Coprinus lagopus* examined by Brefeld (III.)—an advance was then made by separately examining the influence of the different colours of the spectrum on growth. No uniform results, however, were obtained; for, whereas Fischer von Waldheim (I.) found that *Pilobolus cristallinus* was only heliotropic under blue light, both its congener, *Philobolus microsporus*, and *Mucor mucedo* are also sensitive to yellow light, according to Brefeld (IV.) and Regel (I.). The further researches of Wiesner (I.), in contradiction to those of Fischer von Waldheim, show that *Pilobolus cristallinus* and *Coprinus niveus* still continue to turn heliotropically, even in the ultra-red rays. We are indebted to Friedr. Oltmanns (I.) for the settlement of this discrepancy, and also for raising the consideration of this phenomenon to a higher plane than before. His researches were performed on *Phycomyces nitens;* that is to say, the very fungus that had hitherto been regarded as decidedly positive in its heliotropism, and the one chiefly used in lecture demonstrations on account of the unusual sensitiveness of its long sporangial hyphæ to light. By using a very powerful electric arc light (up to 5300 Hefner units), Oltmanns found that the fungus in question behaved positively phototropic under weak illumination, but negatively so under a powerful light, whilst at an intermediate stage of illumination it remained aphototropic. The universal law of stimulants thus applies also to the phototropy of fungi, the sign—to speak mathematically—of the stimulative effect being determined by the strength of the influence. However, the degree of stimulation necessary to the production of a given effect is also dependent on the actual condition of vitality of the individual under examination, age, in particular, being an important factor. Thus, in the case of *Phycomyces nitens*, a given degree of illumination causes attraction in the young sporangial hyphæ (with just grey sporangia), whereas in the older ones (with already blackened sporangia) it induces repulsion. The applicability, to the phototropism of fungi, of Weber's law (§ 233) of the ratio between the degree of stimulation and the effect, has been demonstrated by J. Massart (III.) in the case

of *Phycomyces nitens*. The existence of after effects of stimulation was first remarked, in this connection, by WIESNER (I.).

Although the influence of light on all the other vital manifestations of fungi has been the subject of numerous observations and experiments, no uniform results have been secured, nor has the same elevated point of view been attained as is the case—thanks to Oltmanns—with regard to its influence on the direction of growth. In dealing with this next question, we will follow the natural course of development, and begin with the **influence of light on the germination of the spore**. Now, according to H. HERMANN (II.), or rather E. LOEW (III.), germination is not affected in the case of the conidia of *Penicillium glaucum*, *Tricothecium roseum*, and *Fusarium heterosporium*, or the endospores of *Rhizopus nigricans* (*Mucor stolonifer*). Contrary results were afterwards obtained by VON WETTSTEIN (I.), who found germination retarded by light in the case of spores of *Rhodomyces Kochii;* and by F. ELFVING (I.), who proved that intense sunlight entirely prevents germination in the conidia of *Aspergillus glaucus*.

The reports of experimenters also differ with regard to the **influence of light on the vegetative development**—increase in the size of the cells, and the power of growth. Thus, whereas, according to a report by J. SCHMITZ (I.), *Sphæria carpophila* grows more strongly in the dark than in daylight, *Peziza Fuckeliana*—according to G. WINTER (I.)—ceases to grow in the dark, and perishes entirely if the exclusion of light be prolonged. VAN TIEGHEM (IX.) and GAILLARD (I.) found illumination exercise a favourable influence on the development of *Penicillium* and certain yeasts respectively. KRAUS (I.), on the other hand, found the perithecial hyphæ of *Claviceps microcephala* attain their greatest length in the dark, rather than in red, yellow, or blue light. Brefeld also made the same observation with regard to the stalks of certain species of *Coprinus*. G. H. VINES (I.) found the growth of the sporangial hyphæ of *Phycomyces nitens* prejudicially affected by light, and traced this action to the influence of the blue rays.

The first to investigate the **influence of light on cell fission**, and therefore on **cell reproduction**, was L. KNY (I.), in the case of pressed yeast. He failed to discover any difference in the rate of reproduction in the dark and under moderate illumination by gaslight. This is, however, altered in the case of strong insolation, as will be referred to later on.

Also **in respect of fructification** the individual species of fungi seem to differ with regard to the strength of illumination necessary to the production of a given result; such at least is the conclusion furnished by comparing the reports of various observations conducted on this point, the original probability, based on considerations of a general physiological character,

being heightened by the determinations of Oltmanns. According to H. Hoffmann (II.), E. Loew (III.), and A. Lendner (I.), the formation of sporangia or conidia in *Rhizopus nigricans*, *Thamnidium elegans*, and *Mucor mucedo*, or *Penicillium glaucum* and *Trichothecium roseum* respectively, proceeds just as well by daylight as in the dark. C. Werner (I.) also failed to detect any influence of light on the formation of conidia in two of the higher Ascomycetes. In the case of *Botrytis cinerea*—according to Rindfleisch (I.)—this formation occurs solely by night; and in this instance, as was determined by L. Klein (III.), the retardation is attributable mainly to the blue-violet rays of the spectrum. The converse has been observed in the case of *Rhizopus nigricans*, which, according to A. Lendner, puts forth its sporangia two days later in the dark, or in red and yellow light, than in white, blue, or violet light. In the dark, *Mucor racemosus* produces merely barren sporangia; whilst, in the case of the *Thamnidium aurantiacum* described by Rochard (I.), this fructification is said to proceed most favourably in twilight, and to be prejudiced and retarded by strong light as well as by an absence of illumination. This report, however, has been contradicted both by Payen (I.) and Poggiale (I.). A similar observation to that of Rochard was made by A. Lendner with regard to the formation of conidia in *Aspergillus luteus*, *Asp. niger*, and *Botrytis* (*cinerea?*). Elfving (I.) states that the formation of perithecia in *Aspergillus glaucus* is entirely or to a large extent suppressed by light; and the same applies to the formation of the pileus in certain members of the genus *Coprinus*—congeners of the mushroom—observed by Brefeld (III.).

Nevertheless, the question of the influence of light on the fructification of any given fungus cannot be answered off-hand. On the contrary, it has been established beyond doubt that the nature of the action exerted by light depends on the other conditions of vitality, Brefeld (III.) having shown, in the case of *Coprinus stercorarius*, that the formation of the pileus ceases in the dark when the temperature remains below 15° C. This discovery that light rays can be replaced by heat rays becomes of greater interest when it is borne in mind that—as was determined by Brefeld—the only light rays having any influence on that development are the blue-violet ones, and not the less refractive (*e.g.* the yellow) rays. A determinative influence is also exercised by the composition of the nutrient substratum. Thus, A. Lendner made the observation—which deserves following up—that sporangiation in *Mucor flavidus* occurs in white light, but ceases in yellow or red light or in the dark, when the organism is grown in Raulin's nutrient solution (§ 231); but that the converse is the case when van Tieghem's nutrient solution is used.

Grown on solid media this fungus seems to develop sporangia under any kind of illumination. A fact of not less importance than the **capacity of heat rays to replace light rays**, is that demonstrated by BREFELD (IV.), in the case of *Pilobolus microsporus*, a fungus of the *Mucoracea* family (§ 235), namely, the **after effect of illumination.** When kept in the dark from the beginning, the mycelium of this fungus remains entirely and permanently barren; but if exposed to the light for a couple of hours, and then placed in the dark before any signs of fructification appear, the sporangia will develop, though ordinarily they only do so in the light.

Still more scanty is our knowledge of the **influence of light on the internal life**, *i.e.* the chemico-physiological capacity, of the Eumycetes. In fact it is almost entirely confined to a series of observations made on **respiration** (§ 238), *i.e.* **the exhalation of carbon dioxide.** The first experiments were conducted by WILSON (I.), who failed to discover any influence due to light; and also by BONNIER and MANGIN (I.), and PURIEWITSCH (II.), who experimented with various pileated fungi (*inter alia*, *Agaricus campestris*) and *Phycomyces nitens*, and in all cases found respiration hindered by diffused daylight, the strongest effect being produced by the least refrangible rays. These experiments, however, were of a merely preliminary character, no regard having been paid to the circumstance that the exhaled carbon dioxide may originate from *two* distinct vital processes; viz. either from the conversion of matter in the course of building up the cell, or from the process of combustion within the full-grown cell for the purpose of replacing dissipated internal energy. DUCLAUX (X.) was the first to point out to mycologists this difference, which was already well known in animal physiology. And, as a matter of fact, the subsequent work of ELFVING (I.)—on *Mucor racemosus*, *Aspergillus niger*, *A. flavescens*, and *Penicillium glaucum*—led to the discovery that this differentiation of the two sources of carbon dioxide is also applicable in the case of fungi. In older cultures, wherein the foodstuff is chiefly consumed in order to maintain the vital energy, no apparent influence is exercised by light on the volume of exhaled carbon dioxide. On the other hand, a retarding effect is produced by light in younger cultures, *i.e.* those in which the reproduction and growth of the cells is proceeding briskly. All the above-mentioned workers regarded the total amount of carbon dioxide, liberated by the cultures under observation, as a measure of the respiration; but R. KOLKWITZ (I.) took into consideration, as a by no means negligible source of error in prolonged experiments, the gradual decomposition sustained by oxalic acid under the influence of light (§ 21), since this acid is of frequent occurrence among the metabolic products of fungi. On excluding this source of inaccuracy, by examining

the cultures under a brief illumination by a powerful electric arc, this worker ascertained that considerable acceleration of respiration is experienced in the case of *Oidium lactis*, *Aspergillus niger*, and one species each of *Mucor* and *Penicillium*.

The **action of sunlight** has been the object of a number of observations, which merit our attention the more in that they were chiefly made with yeasts, and partly relate to the biology of the fungoid thorn of the vine. Of the other fungi, *Aspergillus glaucus* was examined by Elfving (I.) in this connection, whereby it appears that the conidia when ripe will stand insolation for several weeks in succession at the latitude of Helsingfors, without injury, though they are killed in a few days when in a young, immature condition. Far inferior powers of resistance were presented by the yeasts subjected to insolation in the south of France by V. Martinand (II.), whose results were afterwards confirmed by G. Tolomei (VII.). It was found that both sporogenic and sporeless cells in two races of the group *Saccharomyces ellipsoideus*, and also cells of *S. apiculatus*, perished after four hours' exposure to the sun's rays at an atmospheric temperature of 41° to 45° C. A similar result followed insolation for three days at 36° to 37° C., whereas other specimens of the same species remained alive when kept in the dark under otherwise equal conditions. This coincides with the results of the experiments made by W. Lohmann (I.) with *Saccharomyces Pastorianus I. Hansen*, two species of *Torula*, two film-yeasts (*Mycoderma*), and a distillery yeast (Race II. of the Berlin Experimental Station), these organisms being killed in a few hours by insolation, as well as by illumination with an electric arc lamp of 8000 to 11,540 metric candle power. The last-named culture yeast succumbed first, whereas the first of the wild yeasts proved the most resistant. The unfavourable conditions artificially produced by Martinand are experienced in practical viticulture by the yeast cells that make their habitat on the grapes most fully exposed to the sun, *i.e.* particularly those on the upper part of the vine-stocks. Consequently we should expect to find in that position a smaller number of living cells than on the grapes lower down. This observation of Martinand's also leads to the conclusion that the grapes from southern countries—which often give very poor fermentation—are less abundantly inhabited by yeast cells, and are chiefly infested by such races as are less sensitive to insolation. It is therefore probable, as a result of this factor of natural selection, that the wine yeasts of southern latitudes are physiologically different from those whose progenitors have lived for centuries under a cooler sky.

An assertion deserving closer investigation is that of Ward (VII.), to the effect that the colouring matters of chromatic fungi afford protection against the injurious influence of light.

Ward supports this view by the personal observation that the colourless spores of *Oidium lactis*, *Chalara myriderma*, and *Saccharomyces pyriformis* were killed by insolation, whereas this was not the case with those of *Aspergillus glaucus*, *Penicillium crustaceum* (*P. glaucum*), *Mucor racemosus*, and *Botrytis cinerea*. It must not, however, be forgotten that the greater power of resistance offered by these spores may well be due to the considerable thickness of the membrane. Nevertheless, as the foregoing discoveries by Elfving have shown, all means of protection fail in presence of prolonged insolation.

With regard to the influence of the Roentgen rays on the vitality of the Eumycetes, an experiment was conducted on *Phycomyces nitens* by L. Errera (I.), but furnished no definite results. Further research on this point is therefore desirable.

§ 223. Chemotropism—General Remarks on the Enzymes of Eumycetes.

Chemotaxis has already been explained (§ 41 of vol. i.) as the attraction or repulsion of motile micro-organisms by chemical stimulants. Motile cells, which are therefore capable of being similarly influenced, are also found among the *Eumycetes*, namely, the *zoospores* (§ 220) of the *Oomycetes* and *Chytridineæ*, i.e. fungi that do not come within the scope of the present work. In the case of the other higher *Eumycetes*, the effects of such a stimulus are manifested by the affected individual either developing with particular strength towards the seat of the stimulus (inclining thereto) or turning in the opposite direction. These phenomena are termed respectively **positive** and **negative chemotropism**. The earliest statements and observations on this point were made by W. Pfeffer (IV.), then by J. Wortmann (VIII.), M. Ward (VI.), M. O. Reinhardt (I.), and others. More thorough investigations were conducted by M. Miyoshi (I.) with *Mucor mucedo*, *Phycomyces nitens*, *Rhizopus nigricans*, *Penicillium glaucum*, and *Aspergillus niger*, in various ways; *inter alia*, by sowing the spores of these fungi on the surface of a finely perforated mica plate, the lower side of which was in contact with the solution of the substance under test. The following were found to act as powerful stimulants on these fungi: ammonium phosphate, among the inorganic substances: and asparagin, dextrin, and various sugars (saccharose and glucose) among organic compounds. Glycerin proved almost entirely inactive.

The minimum quantity of any stimulant capable of producing a chemotactic or chemotropic effect on fungi had already been termed the **marginal limit** (*Schwellenwerth*—literally, "threshold value") by W. Pfeffer. This value was ascertained by Miyoshi

(I.) as 0.01 per cent. in the case of glucose acting on *Mucor mucedo* or *Rhizopus nigricans*. On gradually increasing the dose above this minimum limit, a second limit is reached whereat the effect is one of repulsion, not attraction. Thus, in the case of Miyoshi's experiments, the integument of the spores of *Rhizopus nigricans* just began to be appreciably attracted by a 0.1 per cent. solution of saccharose, the effect then increasing with the dose, until, at about 15 per cent., diminution commenced; and repulsion could be detected at about 50 per cent. The maximum distance at which the influence of the stimulant could be detected was, in the case of the germ tubes of *Rhizopus nigricans* and *Phycomyces nitens*, about twelve to fifteen times the longitudinal diameter of the spores.

Two different explanations have been put forward as to the nature of the intimate causation of negative chemotropism. Massart (I.) assumes that **osmotic action** is really in question, and that consequently the repulsion of the fungus from the seat of the stimulus is a result of **plasmolysis**, due to the high concentration of the solution. On the other hand, Pfeffer (IV.) and Miyoshi found a series of substances which produce an effect of repulsion in all stages of concentration: *e.g.* potassium chlorate, alcohol, all the acids so far examined, &c. Consequently both workers hold the opinion that this effect cannot be explained as the result of osmosis (like the case of the other substances), but is attributable to a specific power of repulsion.

The relation between the strength of the stimulus and the degree of effect produced was investigated by Pfeffer, and found to agree with the general law enunciated by Weber, viz. "The magnitude of the reaction increases in arithmetical progression, as the magnitude of the stimulus increases geometrically." Of course the application of this law is here confined within certain limits, and is therefore excluded when *positive* chemotropism begins to turn into *negative* in consequence of a strongly increased concentration of the stimulant.

Further investigation is merited by the question of how far chemotropic action is concerned in the phenomenon known as **rheotropism**, the outward sign of which is the adoption by fungoid hyphæ of a definite position with respect to the direction of flow of the surrounding liquid. Jœnsson (I.), to whom we are indebted for the first observation on this point, found that the mycelial hyphæ of *Botrytis* grow against the current, but those of *Mucor* and *Phycomyces* with the stream. The first-named fungus he termed positively rheotropic, the other two negatively so.

The tendency of fungoid hyphæ to grow towards a positively chemotropic stimulus is manifested not merely when the latter is freely accessible, but also when it is separated from the influenced fungus by a partition. In some species and under special

conditions, this tendency is expressed by the excretion of substances capable of attacking and perforating the said partitions. Among such substances may be mentioned carbon dioxide and oxalic acid, which come into action more especially when the fungus is in contact with a calcareous substratum, such as an egg-shell or a bone. On this point a number of experiments were conducted by K. LIND (I.) with *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea*.

Of the organic substances naturally forming such a partition wall, viz. either chitin or cellulose, together with allied carbohydrates, the first comes under consideration in all cases where the fungus endeavours to perforate the shell of an insect. In order to effect this it has need of an enzyme capable of dissolving chitin, on which point several reports have been made by ZOPF (X.). However, even when the object infested with the parasitic fungus is a fungus itself, the collaboration of a similar enzyme is necessary, since chitin (§ 226) forms a principal constituent of the membrane of many members of this class. The faculty of excreting an enzyme capable of dissolving cellulose and allied carbohydrates becomes mainly apparent when higher plants are infested by a fungus. The first notice of the occurrence of such an enzyme in *Eumycetes* was that of DE BARY (II.) in 1886, in the case of *Sclerotinia* (*Peziza*) *Libertiana;* and a similar one was recorded in 1888 by M. WARD (I.), in an allied species injurious to lilies. A year later, E. Kissling found the same faculty in *Sclerotinia Fuckeliana*, and this was confirmed by J. BEHRENS (IV.), M. MIYOSHI (II.), and M. NORDHAUSEN (I.). Subsequently, on the basis of his own experiments, M. O. REINHARDT (I.) came to the conclusion that the membrane-dissolving enzyme secreted by the first-named *Sclerotinia* is different from that of the second species. This is by no means surprising, in view of the great variety of carbohydrates (§ 227) taking part in the structure of the vegetable cell wall. The ability of *Botrytis cinerea* to secrete an enzyme capable of dissolving true cellulose has been demonstrated by an experiment, carried out by J. BEHRENS (IX.) in a manner to which no objection can be raised. On the other hand, *Penicillium glaucum*, *P. luteum*, *Rhizopus nigricans*, and probably also *Monilia fructigena*, have been proved incapable, though, with the exception of the last-named species, they can attack the so-called central lamella (§ 119) and therefore secrete a pectin-dissolving enzyme. Another organism capable of dissolving true cellulose is the vine-root mould, examined by J. BEHRENS (XII.), and termed by him *Pseudo-Dematophora*. This is a non-pathogenic mould fungus occupying an unknown position in the botanical system; it grows generally on wood, and has a particular affinity for vine-wood, which it rots and destroys.

According to CZAPEK (II.), the lignified cell walls of higher

plants consist of an ethereal combination of cellulose with a substance to which he gives the name **hadromal**. The dissociation of this compound and the liberation of cellulose, are probably effected by an enzyme which this worker has discovered in certain fungi, *e.g.* dry-rot (§ 80), and which he terms **hadromase**. Fungi that have invaded trees and timber find, in the bark and cambium layer of same, oftentimes large amounts of glucosides, such as salicin, populin, amygdalin, coniferin, &c. Thanks to the action of certain excreted enzymes, such as **emulsin**, they are able to utilise for their own nutrition the carbohydrates separable from these substances. A number of these parasites were examined in this respect, with affirmative results, by E. Bourquelot (III. and IV.). Further mention of Eumycetes enzymes capable of decomposing glucosides will be made, with reference to certain special examples, in a later section.

However useful to parasites may be the faculty of secreting an enzyme capable of dissolving the cell walls of the infested host, there are certain cases where the parasites can dispense with this faculty and still attain the end in view. This is effected by the purely mechanical pressure exerted by the apex of the hypha on the cell wall of the host, when the hypha itself has formed an appressorium (§ 237) and thus provided an abutment. In this manner it succeeds in perforating the cell wall. This faculty has been confirmed by M. Miyoshi (II.) in the case of *Penicillium glaucum* and *Botrytis cinerea*, both of which proved capable of penetrating thin gold leaf quite free from holes. In this case chemical action was entirely precluded. When fungoid hyphæ obtain a footing on the epidermis of such parts of plants as exhibit stomata, they often prefer to obey the chemotropic attraction exercised by the cell contents, in a peculiar manner, by growing towards the nearest stoma, passing, by this means, into the interior of the plant tissue, and then penetrating the cell membranes, which are far thinner than that of the epidermis. An example of this is shown in Fig. 110. The successful entrance of the hyphæ in this manner is not, however, invariably followed by the further development of the parasite, the sequel depending on the constitution of the cell contents. The latter, without prejudice

Fig. 110.—Rhizopus nigricans.

Hyphæ from five spores, which, twenty-seven hours previously, were sown on the under surface of a leaf of *Tradescantia discolor* that had been injected with a 2 per cent. solution of ammonium chloride, making their way to the stoma, and passing there through into the interior of the leaf tissue. Magn. 100. (*After Miyoshi.*)

to their property of attracting the invader, may be of such a character as to preclude its growth. Further information with regard to this biologically interesting and practically important question may be found in handbooks on plant diseases; and a few relevant remarks will also be made later on in connection with the rotting of fruit and "sweet-rot" in grapes.

In the absence of a more favourable opportunity, a few general remarks on the secretion of proteolytic enzymes (§ 170) by Eumycetes may be made here. The first record of a gelatin-dissolving Eumycetes enzyme was that made by Adolf Hansen (I.) in 1889, in cultures of *Mucor mucedo* and *Penicillium glaucum*. Two years later it was shown by A. Weidenbaum (I.) that *Oidium lactis* and *Oidium albicans* differ from one another, inasmuch as the latter does not cause the liquefaction of the gelatinous nutrient medium under any circumstances, whereas liquefaction is produced by the former organism when the medium has an acid reaction. According to Zopf (X.), *Hormodendron cladosporoides* is incapable of secreting such an enzyme. Subsequently, C. Wehmer (XII.) examined a number of mould fungi in this connection, and found that, when grown in a nutrient medium (beer wort, dextrose-nutrient-salt-solution) containing 10 per cent. of gelatin, the following: *Aspergillus niger*, *A. oryzæ*, *A. candidus*, *A. minimus*, *A. Ostianus*, *A. novis*, *Penicillium glaucum*, *P. olivaceum*, *P. italicum*, *P. luteum*, *Botrytis cinerea*, and *Cephalothecium roseum* will liquefy about one half the medium within ten days, and produce complete liquefaction in two to three weeks. The results were indefinite in the case of *Aspergillus glaucus*, *A. fumigatus*, and *A. varians*. According to later reports by the same worker, a slight liquefaction of the gelatin is also effected by *Mucor Rouxii* and *M. javanicus*. In the course of a comprehensive investigation on the occurrence and activity of a gelatin-dissolving enzyme in various members of the vegetable kingdom—including a number of fungi—Fermi and Buscaglioni (I.) obtained affirmative results with sundry edible fungi, as also with *Claviceps purpurea*, *Aspergillus flavus*, &c.

The occurrence of enzymes capable of dissolving casein was investigated by E. Bourquelot and H. Hérissey (I.), who found such a one present in about 20 out of 126 species of fungi examined, *e.g.* in *Amanita muscaria*, *Boletus edulis*, &c. Both workers hold this enzyme to be identical, or at least certainly very nearly allied to, **trypsin**, since, like the latter, it furnishes **tyrosin**. J. Hjort (I.) succeeded in detecting the presence of similar enzymes, capable of digesting fibrin, in various higher fungi; they afterwards completely degrade the peptone formed, from the above substance, along with leucin and tyrosin. Egg albumin is also attacked at the ordinary temperature. Zopf

(X.) has published an observation on fungi capable of dissolving horny matter (keratin).

Diastatic enzymes, *i.e.* such as are capable of converting starch into sugar, are also very often (§ 117) found in *Eumycetes*. Many species of the latter are specially productive in this respect, and are employed on this account in the fermentation industries; this is particularly the case with the species of *Mucor* that will be described in § 240, and the Koji fungus (*Aspergillus oryzæ*) dealt with in the last section but one. At present we shall not treat of the technico-mycological side of this faculty, but merely make a remark of a general physiological character, namely with regard to the dependence of the formation of this enzyme on the external conditions. In this connection W. Pfeffer (V.) and Jul. Katz (I.) examined *Penicillium glaucum*, *Aspergillus niger*, and *Bacillus megatherium*, and found that these two Eumycetes produce diastase, even in the absence of starch, provided no adverse influence comes into play. Such an influence has been traced to the presence of various sugars, in the case of *Penicillium glaucum*, the formation of diastase at medium temperatures ceasing when the (otherwise identical) nutrient media received an addition of either 1.5 per cent. of saccharose, 2 per cent. of grape sugar, or 10 per cent. of galactose. The secretion was also retarded by 5 per cent. of maltose. Enriching the nutrient properties of the medium is succeeded by an increase in the limit of the foregoing additions. In the case of *Aspergillus niger*, an addition of even 30 per cent. of saccharose merely restricts the formation of diastase (at 31.5° C.) without suppressing it altogether. The amount of diastase secreted per unit of time by this fungus was found to be greater (other conditions being equal) in the case of cultures in which provision had been made for the immediate separation of the resulting diastase by additions of tannin.

Enzymes (lipases—Hanriot (I.)—or steapsines—W. Biedermann (I.)) **capable of decomposing fats**, are found not only in the pancreas, blood, and other corporeal fluids of all animals hitherto examined, but also in the vegetable kingdom. Their occurrence in *Eumycetes* was first detected in the case of *Penicillium glaucum*, the discovery being made by E. Gérard (II.) and L. Camus (I.). They were then found by the last named (II.) in *Aspergillus niger*, and by R. H. Biffen (I.) in an unidentified fungus infesting the cocoanut. J. Hanus and A. Stocky (I.) reported having observed the production of a similar enzyme by a number of mould fungi in the course of their investigations—referred to in a later section—on mouldiness in butter. It is not improbable that several varieties of lipases exist in the fungoid kingdom. The faculty of producing such an enzyme is of particular importance when the nutrient medium is rich in fat, *e.g.* in the case of fungoid parasites

infesting the fatty matter of insects. This faculty may be regarded as non-essential to such fungi as make their habitat on oilcake (§ 235) and consume the fat therein, since—as has been shown, particularly by W. Sigmund (I.)—the oil seeds frequently contain lipase, and it is very probable that some portion of this is left behind in the oilcake. It is, however, doubtful whether this residue has not already lost its power, and is consequently incapable of being utilised by the infesting organisms, the lipases being very sensitive to injurious influences (acids, salts, &c.) For this reason, it may be remarked in passing, all attempts at their isolation in a pure state have failed. The capacity of producing fat-decomposing enzymes is certainly possessed by the fungi which, within a few months, reduce the fat content in the mud of settling tanks in waste-water purifying plant, from over 20 per cent. to almost nil. Sundry highly instructive experiments on this point have been conducted by J. Bechold (I.).

With regard to the various enzymes of yeast in particular, full details will be given in a later section.

SECTION XI.

FERMENTATION BY ZYGOMYCETES.

CHAPTER XLIII.

MORPHOLOGY AND SYSTEMATOLOGY OF THE MUCORS.

§ 234.—Subdivision of the Order of Zygomycetes.

The Order of *Zygomycetes* (§ 221) is characterised by the capacity of forming **zygospores**. According as these resting cells are able to envelop themselves in a more or less completely developed capsule, or remain naked, the corresponding **Zygomycetes** are divided into two sub-groups. The higher of these, viz. the one forming the spore capsules, has received the name, "**carposporangial**," from Brefeld, who united the whole of the members into a family, a few particulars of which will be given in § 237. Antithetical to these are the remaining, or "**exosporangial**," *Zygomycetes*, as they were termed by Brefeld. It is with this (larger) sub-group, the sporogenic organs of which remain naked, that we have first to deal.

The occurrence of several **methods of fructification** in one and the same species of fungus has already been mentioned in § 223, and is specially prevalent among the *Zygomycetes*. Apart from the characteristic zygospores, they are able to ensure the reproduction of the individual in other ways, special aptitude in this direction being exhibited by the genus *Choanephora*, which, in addition to zygospores and gemmæ, also puts forth conidia and endospores. In all other *Zygomycetes* only one or other of the two last-named methods of fructification is exhibited by any one individual. Sporangial fructification is characteristic of the *Mucor* family, a number of examples of which will be more closely considered later on.

The other *Zygomycetes*, on the contrary, exhibit conidial fructification, and are divided into three families, which do not come directly within the scope of the present work, and will therefore be dismissed with a few brief explanatory remarks. The family of the **Entomophthoreæ** forms a connecting link between the orders of *Zygomycetes* and *Oomycetes*. The species of this family are almost exclusively parasitic on living organisms (insects, fungi, ferns), and one of them will, from its effects at any rate, be, superficially, known to the reader, namely, *Empusa*

muscæ, the cause of a disease attacking the house-fly in late summer and autumn. From this cause numbers of these insects are found to adhere, straddle-legged, to walls and windows, and become surrounded by a distinct white aureole, consisting of unicellular conidia dispersed by the conidiophores protruding from the body of the insect. These conidia germinate into a structure similar to a budding mycelium (§ 219); and it is owing to this faculty that the wraith of the house-fly has for so long made its appearance in the controversy on the origin of the *Saccharomycetes* (§ 344).

The second of the three families of conidiophorous *Zygomycetes*, namely, the **Piptocephalideæ**, on the other hand, lacks the faculty of producing budding cells (like the third family), and is distinguishable by the feature that the zygospore apparatus consists of five cells instead of three, as also by the fact that the conidia are multicellular. The members of this family are parasitic on various species of *Mucor*, to the hyphæ of which they attach themselves by means of special organs (haustoria), and then penetrate the interior for the purpose of abstracting nourishment. This behaviour they exhibit in common with the species of the third family of conidiophorous *Zygomycetes*, namely, the **Chætocladiaceæ**, whose conidia are unicellular and may be regarded as sporangia, whilst the contents are united to form a single endospore, instead of being divided into several. By this feature they reveal the connection, as regards progress of development, between conidial and sporangial fructification.

We can now collect the above particulars into the form of a

SCHEMATIC SUBDIVISION OF THE ORDER OF ZYGOMYCETES.

- **Zygomycetes**: *Phycomycetes* capable of producing zygospores
  - (*a*) Naked zygospores
    - (*α*) Exhibiting sporangial fructification, but lacking conidial fructification . . . — *Mucoraceæ.*
    - (*β*) Exhibiting conidial fructification, but lacking sporangial fructification . . . — *Entomophthoreæ.*, *Piptocephalideæ.*, *Chætocladiaceæ.*
    - (*γ*) Exhibiting both sporangial and conidial fructification . . . — *Choanephoreæ.*
  - (*b*) Zygospores enclosed in a capsule . . — *Carposporangial Zygomycetes.*

Of these six families, only the first and last will be dealt with in the following paragraphs, chiefly from the standpoint of Physiology and the technology of fermentation, leaving out of consideration their morphology and development except in so far as mention of these is absolutely essential to our purpose. Readers desirous of obtaining fuller information on the two latter points are referred to ALFRED FISCHER'S (III.) monograph on the *Phycomycetes* and *Zygomycetes*.

§ 235.—Subdivision of the Mucor family.

From the explanations in the preceding paragraphs, the *Mucoraceæ* may be defined as *Zygomycetes* which exhibit sporangial, but not conidial, fructification, and produce naked zygospores. This family may be subdivided into three sub-families, of which, however, only one falls within the scope of the present work, whilst the others will merely be referred to in order to facilitate comprehension of the connection existing between them.

The sub-family of **Thamnidieæ** is characterised by the possession of two kinds of sporangia: on the one hand, a large, normal, polysporous sporangium, on the crown of the sporangiophore (terminal sporangium); and, on the other hand, certain far smaller sporangia, which are situated lower down and put forth by whorled lateral branches of the sporangiophore. This second kind of sporangia are destitute of columella, contain only a pair of endospores—sometimes only a single one—and are known as sporangioles. The most closely investigated member of this sub-family, *Thamnidium elegans*, is shown in Fig. 111. Starting from observations conducted by Brefeld (IX.), the dependence of fructification, in this species, on the external conditions (chemical composition and concentration of the nutrient substratum; temperature) was examined by J. Bachmann (I.). This worker demonstrated that, by controlling these conditions, it is possible to compel the fungus to produce either terminal sporangia or sporangioles exclusively, or both together, or again to remain barren of fruit.

The second sub-family of the *Mucoraceæ*, namely, the **Piloboleæ**, is distinguished by the feature that the ripe sporangium—owing to its peculiar structure—is released, and even forcibly expelled from, the organ on which it has been developed. Of the species belonging to this group, mention may be made of *Pilobolus crystallinus*, which is often found on horse droppings.

Finally, the **Mucoreæ** constitute the third sub-family. They are distinguishable from the first by producing only a single kind of sporangia, which, unlike those of the second sub-family, do not separate from the sporangiophore before discharging their contents, but remain attached thereto after bursting. The discharge is effected in consequence of either the liquefaction or brittleness of the membrane of the ripe sporangium.

One of the genera in this third family, viz. the genus *Sporodinia*, is characterised by the forked branchings of its sporangiophores and the suspensores of the zygospores. This form is represented in Fig. 107. On the other hand, in all the remaining *Mucoreæ*—which have been arranged into five genera by A. Fischer (III.)—these organs are either not branched at all or at least not forked.

FIG. 111.—Thamnidium elegans Link.

1. Sporangiophore, slightly (6) magnified ;
2. Three pieces of same, more highly (120) magnified ; *a*, terminal sporangium ; *c*, sporangioles.
3. Stunted sporangiophore, exhibiting only sporangioles. Magn. 200.
4. Sporangioles that have separated from the sporangiophore. Magn. 200. (*After Brefeld.*)

The genus *Phycomyces* is characterised by the possession of spinous prolongations on the suspensores and sporangiophores, which latter are unbranched, olive-green in colour, and possess metallic lustre.

One species of this genus, *Phycomyces nitens*, is plentifully met with in empty oil-casks, on oil-cakes, in concentrated fodder works, and similar places, and puts forth stiff, upright sporangiophores 7 to 30 c.m. long and 50 to 150 μ in diameter. These become crowned with an initially orange, but finally black, globular sporangium, 0.25 to 1.0 m.m. across, exhibiting a cylindrical columella and filled with ellipsoidal, yellow-brown, thick-walled endospores, 16 to 30 μ long and 8 to 15 μ broad. It has not yet been ascertained whether this species feeds on the fats present in the aforesaid medium; in fact, the whole question of the decomposition of fats by mould fungi (§ 233), on which sundry observations have been communicated by R. H. Schmidt I.) and by Ritthausen and Baumann (II.), is a matter still requiring closer attention.

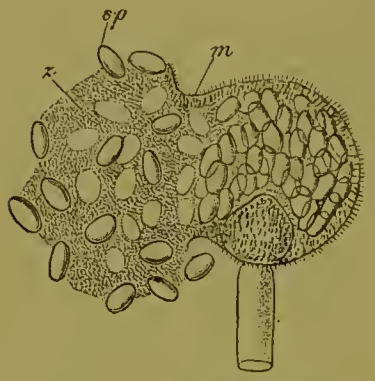


Fig. 112.—Mucor mucilagineus Brefeld.

Newly burst sporangium. *m* is the membrane, *z* the matrix, *sp* the spores. Several of the latter have been squeezed out of the sporangium. Magn. 300. (*After Brefeld.*)

The genus *Mucor* is distinguished by the absence of spiny branches on the suspensores, by the silky gloss of the sporangiophores, and by the liquefaction of the ripe sporangium membrane. The contents of the ripe sporangium are only partly consumed in building up the spores, the remainder serving as a matrix wherein the individual ripe spores are embedded and separated from each other. Now this matrix is capable of distension, and holds the crowd of spores together, even in microscopical preparations; whereas on the other hand the sporangial membrane, being liquefiable, is either quite invisible in such (aqueous) preparations, or at most is only seen as a residual trace at the point of attachment to the sporangiophore. An attempt is made to portray this in Fig. 112. The outside of the sporangial membrane is found to be more or less closely set with crystals of calcium oxalate. A few of the species composing this genus are pathogenic, and therefore interesting to the pathologist, since they are able to set up mycosis (in this case *Mucor-mycosis*) or fungification of the body they have infested, or into which they have been artificially inoculated. Of these pathogenic species of Mucor—some of which have not yet been properly examined and classified in a botanical sense—mention may be made of the following: *Mucor pusillus*, discovered by

Lindt; *Mucor corymbifer*, examined by Lichtheim, and which is probably identical with Lindt's *Mucor racemosus*, subsequently also named *Rhizopus ramosus;* finally Siebenmann's *Mucor septatus*, which is perhaps the same as *Mucor racemosus*. These pathogenic species have all been tried on warm-blooded animals, and therefore thrive at incubation temperature.

§ 236.—The Genus Mucor

was established by Micheli as far back as 1729. During the succeeding 140 years it received attention at the hands of a large number of workers, and a considerable number of species have been described — for the most part imperfectly. The species of this genus may be divided into three groups. One of them comprises all the species with **unbranched sporangiophores**, the chief representative being *Mucor mucedo*, with which is associated *M. mucilagineus*, &c. The species of the second group may exhibit **clustered branchings** of the sporangiophores; they include *Mucor racemosus*, *M. erectus*, *M. tenuis*, *M. fragilis*, *M. corymbifer*, and *M. pusillus*. Finally, the characteristic feature of the third group is a more or less decided **sympodial branching** of the sporangiophore. This group comprises *Mucor spinosus*, *M. circinelloides*, *M. alternans*, as well as the *M. Rouxii*, *M. javanicus*, &c., described in § 240. The former will now be described from a morphological standpoint, their physiological characteristics being deferred for consideration until § 239.

Mucor mucedo is the oldest known species, and was first fully described by O. Brefeld (I.) in 1872. The development of its mycelium has already been diagrammatically illustrated on p. 2. From the mycelium itself arise stiff sporangiophores, 30 to 40 μ thick and (according to the conditions of growth) 2 to 15 c.m. in height. The apex of each supports a single globular sporangium, which is closely covered with fine needles of calcium oxalate, and usually measures 100 to 200 μ in diameter, though, as shown in Fig. 113, it may be much smaller under unfavourable conditions. The spores, which are in the shape of an elongated ellipsoid, and about twice as long as they are broad, may differ in size in one and the same sporangium, the usual measurements, however, being 6 to 12 μ and 3 to 6 μ respectively. The cell contents are faint yellow in colour, the membrane colourless and smooth. The zygospores, the gradual development of which is represented in Fig. 101, germinate by putting forth direct an unbranched sporangiophore with an attached sporangium. This species does not produce gemmæ.

A sporangium of *Mucor mucilagineus*—which was first mentioned by Brefeld (IV.)—is shown in Fig. 112, which also reveals the considerable dimensions of the spores. These are

oval, 30 to 33 μ long and 15 μ broad. It has an affinity for horse-droppings, and is revealed, in cultures on this medium, by the surface of the sporangiophore being thickly covered with fine drops, as though bedewed.

Mucor racemosus Fresenius exhibits not merely the clustered branching of the sporangiophore, shown in Fig. 114, and a somewhat different development of the columella, but also the formation of chlamydospores already mentioned in § 223. These are not peculiar to this species, but are found on several allied kinds, though in others they are entirely lacking. Consequently, BREFELD (VIII.), to whom we are indebted for thoroughgoing researches on this point, united the former into a new genus, *Chlamydomucor*; and on this account *Mucor racemosus* also appears in the literature, under the synonymous designation, *Chlamydomucor racemosus*. It produces sporangiophores, which, according to the environment, measure 5 to 40 m.m. in

FIG. 113.—Mucor mucedo Brefeld.

1. Sporangium of normal dimensions. *m*, the membrane; *sp* the spores; *c*, the columella. Magn. 225.

2. Residue of an emptied sporangium. Only a fragment (*m*) of the membrane is left on its point of attachment to the sporangiophore; *c* is the columella. Magn. 180.

3. Stunted dwarf sporangia, with only a few spores and devoid of columella. Taken from an individual infested with a parasitic fungus (Piptocephalis). Magn. 300. (After Brefeld.)

height and 8 to 20 μ thick. Each of the several branches is crowned with a sporangium, 20 to 70 μ in diameter. The spores are globular, or of short ellipsoid form, and measure 6 to 10 μ in length by 5 to 8 μ in breadth. Fuller particulars respecting allied species, many of which are probably nothing but sports, will be given in §§ 240 and 241.

Mucor spinosus Rabenhorst has already been mentioned (§ 221) as an instance of a fungus forming azygospores in addition to zygospores. As there shown in two Figs., sporogenation proceeds in this species in a manner similar to the typical formation of zygospores, in so far as two opposite gametes are produced.

This procedure is more decidedly evident in the case of *Mucor Mucedo Rabenhorst*, there being no approximation of the pairs of

sporangiophores. The azygospores are formed, singly and independently, on the apices of branches of an erect sporangiophore, arising from the nutrient substratum (or the mycelium), and which therefore assumes a clustered appearance. It is represented in Fig. 115. On the other hand, normal zygospores have not hitherto been discovered on this fungus.

Mucor fragilis Bainier resembles the foregoing species as regards the structure of the sporangium; except that the latter is smaller. The same applies also to the spores, which are oval in shape, about 4 μ in length, and 2 μ in breadth.

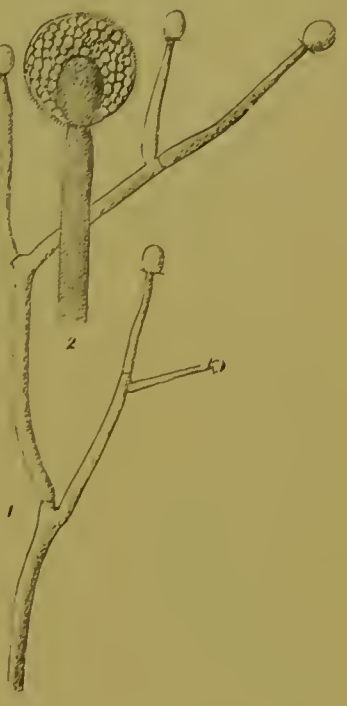


Fig. 114.—Chlamydomucor racemosus Brefeld.
1. Branched sporangiophore. Magn. 80.
2. Optical section of sporangium, more highly magnified (300 times). (*After Brefeld.*)

Mucor spinosus van Tieghem owes its specific name to the spiny projections, exhibited, to the number of about a dozen, on the crown of the cylindrical or pearshaped columella. In preparations submerged in water, the membrane quickly dissolves and vanishes from sight, leaving a picture recalling that of the head of a conidiophore of any *Aspergillus*. On this account the fungus was at one time called *Mucor aspergilloides*, though the name has now gone out of use.

Mucor circinelloides van Tieghem, and *Mucor alternans van Tieghem*, are very similar in a morphological sense, particulars on which point will be found detailed by Alf. Fischer (III.). The former of the two has been drawn by Gayon (VI.), and the other by Gayon and Dubourg (III.). Morphologically, both species form connecting links between the allied genera *Mucor* and *Circinella*, which latter differs chiefly from the former by the drooping manner in which the sporangia overhang and by the resistance of their membrane.

Mucor piriformis, which was first discovered (on rotten apples) and described by Alfred Fischer (III.), was so called on account of the pear-like shape of the columella, the broader (about 140 to 280 μ) upper end of which extends a distance of about 200 to 300 μ within the sporangium. When light is admitted to cultures of this fungus, grown in a suitable nutrient solution, it elaborates comparatively large quantities of citric acid from

sugars, as was pointed out by C. Wehmer (X.). In fact a patent has been taken out in Germany (No. 91,891) for a process of manufacturing citric acid by this means. The subject will be mentioned again in a later chapter.

§ 237.—Rhizopeæ.

The higher, or **carposporangial, group** of the *Zygomycetes* (§ 234) can be divided into two families: **Rhizopeæ** and **Mortierellæ**. The former of these exhibits in a lower degree a peculiarity more highly developed in the second family, namely, the formation of an integument, which surrounds the sporogenic organ and therefore raises it to the dignity of a true spore fruit. This second family does not come within our purview, and all that need be said about it is that all its species, especially *Mortierella Rostafinskii*—which occurs on horse-dung and was discovered and closely examined by Brefeld (IV. and IX.)—exhibit this method of fructification. From the earliest period of their development the zygospores are surrounded by a progressively increasing network of hyphæ, which spring from the adjacent mycelium, branch extensively, and finally weave themselves into a felted capsule surrounding the zygospore on all sides. A less extensive development is exhibited by the integument, which endeavours to envelop the sporangium; it does not increase to more than a dense cluster of hyphæ, which surround the lower end of the sporangiophore, without, however, being able to reach the sporangium itself. A view of this is given in Fig. 116.

G. 115—Mucor tenuis Bainier.
Azygospores in various stages of development. (*After Bainier.*)

In the family *Rhizopeæ*, however, which comprises the two genera *Rhizopus* and *Absidia*, this integument of the zygospores is either absent (*Rhizopus*) or only slightly developed (*Absidia*), being arrested at the stage of spiny processes protruding from the suspensores. The special position of this family, outside the exosporangial *Zygomycetes* (§ 234), forming as it does the connecting link between them and the *Mortierella*, cannot therefore be founded on this indefinite characteristic of integumentation, but rather depends on the property it shares with the family just mentioned, namely, the development of stolons and the resulting peculiar conformation of the thallus. Whereas in the *Mucoreæ*,

which closely resemble the *Rhizopeæ* in many other respects, the sporangiophores spring from parts of the mycelium that are not in any way specially distinguishable from the rest, there is developed between these two organs, in the *Mortierella* and *Rhizopeæ*, an intermediate organ which determines the location of the sporangiophore. This is effected in the following manner: a hypha, which, from its peculiarities, is called a **stolon**, begins to project outward from the mycelium growing in and upon the nutrient substratum. Describing a considerable curve, this stolon endeavours to make its way to a substratum, which may either be a distant portion of the nutrient medium, or the walls of the vessel in which the latter is contained. The peculiar nutational movement described by the apex of the growing stolon, was more closely examined by J. Wortmann (XIV.) in the case of *Rhizopus nigricans*. On coming into contact with the desired substratum, the stolon first endeavours to attach itself thereto by putting forth a system of short hyphæ, which fit close against the substratum, and on account of their branched and root-like form are termed **rhizoids**. In their entirety they constitute an adhesive organ, called an **appressorium**, which is copiously supplied by the stolon with nutrient materials from the distant mycelium, and in turn puts forth stolons of the second order. These now extend to farther distances and produce appressoria of the second order, which form the starting point for stolons of the third order, and so on; and in this manner the vicinity of the nutrient substratum is overrun with a fine network extending over a considerable area. It is from this network, and not from the mycelium itself, that the sporangiophores proceed, springing from well-defined centres. Thus, in the case of the genus *Rhizopus*, the sporangiophores take their rise exclusively from the appressoria, from each of which spring usually three to five, more rarely up to as many as ten. In the genus

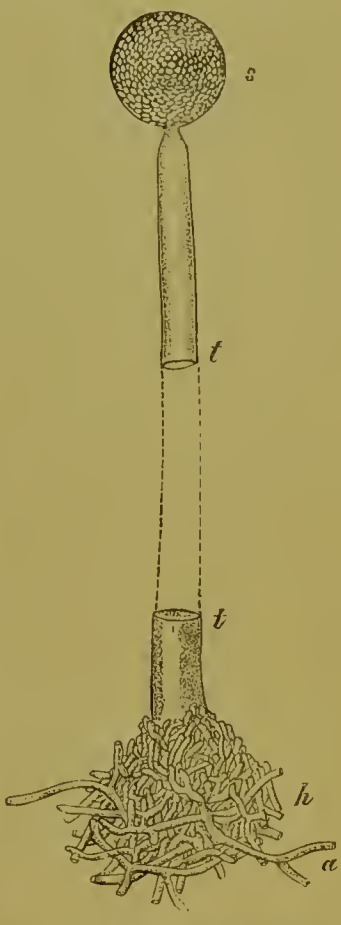


Fig. 116.—Mortierella Rostafinskii Brefeld.

The lower end of the sporangiophore (*t*) is enveloped by a plexus of hyphæ (*h*), which have proceeded from the stolon (*a*). Magn. 100. (*After Brefeld.*)

Absidia, on the other hand, the sporangiophores spring from the crown of the arch formed by the curved stolon.

The development of appressoria is a consequence of the mechanical attraction exerted on the tip of the stolon by the objects with which it comes in contact. If means be taken

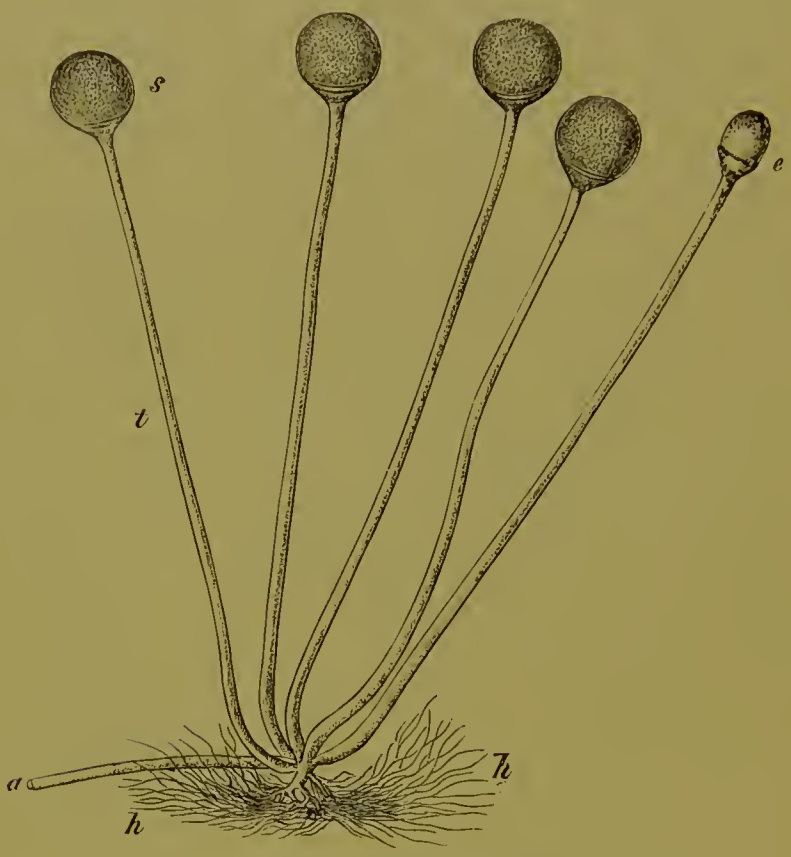


FIG. 117.—Rhizopus nigricans Ehrenberg.

(*a*) is the extremity of a stolon, which has developed into the appressorium (*h*). This latter is the starting point of the sporangiophores (*t*), four of which are shown with the sporangia (*s*) unbroken, whilst the columella (*e*) is all that remains of the fifth. Magn. 30. (*After Brefeld.*)

to prevent the occurrence of such contact, for example by compelling the stolon to grow vertically downwards, the stolon itself will develop into a sporangiophore direct. Instead of forming an appressorium, the apex—as J. WORTMANN (XIV.) has shown in the case of *Rhizopus nigricans* — develops into a normal sporangium. Another instance, in the fungoid kingdom, of the effect of such contact attraction—which is also frequently observed in the higher plants, especially in the curvature of tendrils—is met with in *Phycomyces nitens*, and was first noticed by ERRERA (VIII.), but afterwards more fully

investigated by Wortmann (XIII.). The sporangiophores of this *Mucorea* when gently touched at the side, curve in such a manner that the place touched assumes a concave form. This phenomenon is termed **haptotropism.** We are indebted to A. de Bary (II.) for the earliest observations on the relation between contact attraction and the formation of appressoria in fungi. The matter afterwards engaged the attention of M. Buesgen (II.)—in the case of *Botrytis cinerea*—and others.

Rhizopus nigricans is the best and longest known member of this family. In 1818 it was described by Ehrenberg under the name *Mucor stolonifer*, which is still used by several workers; and the same authority afterwards conferred on it the new appellation. This saprophyte readily and almost invariably infests vegetable substrata—*e.g.* fruit—when the latter have been exposed to the air for some time in a damp condition. The stolons often attain a length of 3 c.m.; the sporangiophores may grow to a height of 4 m.m. (Fig. 117). The sporangium is pure white at first, afterwards yellow, and finally almost black. When ripe, the membrane liquefies on coming in contact with water, and therefore ceases to be visible in ordinary microscopical preparations. In such case, beginners generally mistake the large columella for the wall of the sporangium. The spores are of varied form, mostly oval, and their longitudinal measurement is 6 to 17 μ.

The name *Rhizopus oryzæ* has been given by Went and Prinsen Geerligs (I.) to a fungus discovered by them in Ragi (241), the sporangia and spore of which organism are considerably smaller than those of *R. nigricans*. Nevertheless, fluctuations in size are met with in both cases, according to the conditions of nutrition and development. This species was examined by C. Wehmer (XIV.), who failed, however, to find any fundamental difference between the same and *R. nigricans*; and it is therefore probably nothing more than a variety of this last fungus.

CHAPTER XLIV.

FERMENTATION BY MUCORS.

§ 238.—Intramolecular Respiration and Alcoholic Fermentation.

The exhalation of carbon dioxide, to which the name **respiration** has been given, may occur, in both animal and vegetable cells, under two widely different sets of conditions: either in the presence and unrestricted access of oxygen, or in the entire absence of this gas. In the former case the process is termed oxygen respiration; but only a few remarks can here be made in connection therewith, for the most part to supplement the thorough description given in Pfeffer's (III.) *Handbook of Vegetable Physiology*. The volumetric ratio between the absorbed oxygen and the evolved carbon dioxide, or, as it is termed, the **quotient of respiration** ($CO_2 : O_2$), was found by Saussure, as long ago as 1833, to vary with the nature and actual condition of the plant under examination, as well as with the conditions of nutrition. With regard to this latter factor, it was shown by Diakonow (II.), in the case of *Penicillium glaucum*, that—other conditions being equal—the quotient of respiration is equal to unity when the foodstuff is sugar, but 2.9 when tartaric acid forms the nutrient substance. Puriewitsch (III.) then demonstrated, in the case of *Aspergillus niger*, that an increase in the concentration of the nutrient solution causes the quotient to rise at first, but afterwards to recede. The influence of temperature on the quotient of respiration was investigated by C. Gerber (I.) in cultures of the last-named fungus grown in Raulin's nutrient solution, with an addition of tartaric (malic or citric) acid, either alone or in conjunction with saccharose, as the organic foodstuff. Mention has already been made in § 233 of the influence of the degree of illumination on the quotient of respiration, as well as on a few other points in connection therewith.

We shall have to deal in a more thoroughgoing manner with the second kind of respiration, to which the name of **intramolecular respiration** has been given in consequence of a proposal made by Pflueger in 1875. This phenomenon consists of the persistent exhalation of carbon dioxide by cells excluded from a supply of free oxygen. The sole materials for this

respiration are the carbohydrates, and in fact, primarily and directly, only certain kinds of sugar, which are decomposed in such a manner as to furnish, in addition to carbon dioxide, a residual product poorer in oxygen. This product, however, is by no means a uniform entity, but consists of a mixture of several substances, alcohol being an invariable constituent. In higher plants the energy with which carbon dioxide is liberated during intramolecular respiration is not infrequently on a par with that exhibited in normal respiration, and indeed is in some cases even superior; though, as has been shown by WILSON (I.), it is always inferior thereto in the case of fungi. All the preliminary conditions necessary to the inception of such a process of decomposition are present in the case of sweet (germ-free) fruits that are kept, uninjured, for a long time out of contact with air; and, in fact, it was in connection with such fruits that the first observations on intramolecular respiration were made, early in the nineteenth century. At that time the phenomenon was known as the **autofermentation of fruit** (see § 20). The extensive literature of this subject has been collected in a meritorious treatise by C. GERBER (I.). It is not within the province of the present work to go more closely into the botanico-physiological aspect of this phenomenon; and besides it will be found thoroughly reported in the above-mentioned *Handbook* of Pfeffer's, by all who are interested in the matter.

The first observation of intramolecular respiration in fungi was that of BAIL (I.), who, in 1857, showed that the mycelium of certain (unspecified) *Mucors*, when kept submerged in a saccharine liquid and out of contact with air, assumes an unusual form (see § 219), and instead of consuming the sugar to carbon dioxide, as it does in presence of air, converts the same in such a manner as to produce alcohol. This new fact was seized upon by PASTEUR (II.), who endeavoured to utilise it as a pillar of support for his novel theory of fermentation. This we have already referred to in § 16 and § 113, mentioning at the time that it is not universally applicable. In fact it is only unconditionally true in the case of a single group of phenomena, namely those produced by strictly anærobic agents, the butyric-acid bacteria in particular. On the other hand, difficulties are encountered in the case of alcoholic fermentation, NÆGELI (III.) having shown that this fermentation, when excited by yeasts, proceeds just as well, or even better, in presence of free oxygen as when that gas is excluded—a result entirely opposed to the theory in question. Nevertheless, the observations of Pasteur and his predecessors, concerning the production of alcohol by *Mucoreæ* under the specified conditions, remain unimpeached; only, this formation of alcohol must not be regarded as perfectly identical in nature with the alcoholic fermentation excited by yeasts.

Mention will be made further on respecting the difference between the two processes.

As already hinted, the presence and accessibility of certain kinds of sugar must be regarded as an indispensable preliminary condition for the occurrence of intramolecular respiration; and this will be more fully elucidated in subsequent paragraphs. Even under otherwise equal conditions, differences exist among the various fungi in respect of the extent to which intramolecular respiration may develop, and of the energy with which the resulting liberation of carbon dioxide proceeds. In some species the phenomenon seems almost entirely absent, and in others, *e.g. Rhizopus nigricans*, it is very feeble. According to Diakonow (I.), *Penicillium glaucum* and *Aspergillus niger* cease to give off carbon dioxide very soon after the exclusion of air has been effected, and they perish within an hour, even when grown in nutrient solutions containing sugar. In this case, as was shown by Pasteur (III.) and Brefeld (XIII.), merely extremely minute traces of alcohol are formed. According to a private communication from Prof. Hansen, Schiœnning has failed to confirm the contrary report of Elfving (I.) to the effect that he found considerable amounts (up to 4.2 per cent. by vol.) of alcohol in cultures of the first-named fungus; consequently this report must undoubtedly be based on error. Apart from the true alcohol-yeasts, in the restricted sense of the term, the producers of the largest (percentage) quantities of alcohol are to be found among the *Mucoreæ*. The behaviour of these under the conditions inducing intramolecular respiration will be dealt with in the next paragraph.

We will now turn to the difference between intramolecular respiration and **alcoholic fermentation**, and ask ourselves whether this difference is one of principle or merely of degree. Those who inclined to favour the latter hypothesis were able to advance many circumstances in its favour. The chief products (alcohol and carbon dioxide) and the subsidiary ones (glycerin and succinic acid) are the same in both decomposition processes. The objection raised by the opposite party, namely that the ratio of transposition is different in intramolecular respiration, and that a smaller quantity of alcohol is formed per unit weight of sugar consumed, was disposed of by the discovery of Godlewski and Polzeniusz (I.), in 1897, to the effect that peas stored out of contact with air produce 101 to 103 parts of alcohol to 100 parts of carbon dioxide exhaled, *i.e.* nearly as much as the 104.5 per cent. that should result from alcoholic fermentation according to Gay-Lussac's equation. Nevertheless, so far as we are able to judge in the present state of our knowledge, there exist two fundamental differences between the processes in question. One of them has already been mentioned, namely the inception of alcoholic fermentation by yeasts in presence of air; and the

second will become apparent as the mechanics of the two phenomena are examined and compared. As observed in higher plants and *Mucoreæ*, intramolecular respiration is undoubtedly an intracellular process, a manifestation of vitality on the part of the cell as such, and therefore inseparable from the corpus of the cell. On the other hand such a local limitation cannot be assumed in the case of alcoholic fermentation by yeast since it has become known that this phenomenon is not due to the action of the cell *per se*, but to a contained enzyme that is able to exert its powers outside, and independent of, the cell in which it originated, and can incite the aforesaid decomposition of sugar when placed in a test-tube. Through the discovery of this new fact, the phenomenon of alcoholic fermentation by yeast has lost, at one blow, its character as a direct manifestation of vitality on the part of the yeast cell, and has been transferred to the category of enzymatic action. Consequently, those who defend the hypothesis that these two processes are identical in principle, and differ merely in degree, will have to seek for the concomitant action of a similar enzyme in intramolecular respiration as well. So long, however, as this has not been proved we must still uphold the assumption that, on the basis of facts—so far as these are known—a distinction must be drawn between the formation of alcohol by intramolecular respiration (proceeding entirely within the cells themselves) and the alcoholic fermentation excited by a yeast enzyme (which may also act outside the cells). Nevertheless, for the sake of simplicity, we may still continue to speak in the following paragraphs of alcoholic fermentation induced by *Mucoreæ*, provided the difference specified be borne constantly in mind.

§ 239.—Mucor Fermentations.

Bail, who discovered that alcohol is formed by intramolecular respiration in certain species of *Mucors*, did not further investigate the chemico-physiological aspect of the question. Furthermore, the researches of Pasteur (III.), the next labourer in this field, suffer from the defect that he did not work with pure cultures, without which here (as elsewhere) no reliable results can be obtained ; and besides, these experiments were mainly undertaken for the sole purpose of supporting the new theory of fermentation. Hence Fitz (IX.) was really the first (in 1873) to examine the question from the chemical side, *Mucor racemosus* being the organism selected for the purpose. Then followed U. Gayon (V.), who in 1878 tested *Mucor spinosus* and *M. circinelloides* for the possession of the faculty in question. Undeniably pure cultures, however, were first employed by E. Ch. Hansen (VIII.), who in 1887 reported on his experiments with *Mucor mucedo*, *M. racemosus*, *M. erectus*, and *M. spinosus*. His dis-

coveries were afterwards supplemented by other workers who will be mentioned later on.

The degree of **susceptibility towards alcohol** is an important factor in solving the question as to the suitability of these fungi for the purposes of the distiller, since it is this sensitivity which, other conditions being equal, determines the **attainable production of alcohol.** Another influential factor is the fermentative activity, *i.e.* the duration of time in which a given quantity of alcohol is produced. A few reports on this point are collected below :—

Mucor mucedo has produced :—

According to	After	In	Alcohol
PASTEUR (III.) . .	14 months	wort	1.8 % by weight
BREFELD (XV.) . .	...	...	2.6 % ,,
FITZ (XI.) at 30° C. .	7 weeks	must	0.8 % ,,
HANSEN (VIII.) at 23° C.	15 days	wort	0.5 % by volume
and at room temperature	6 months	wort	3.0 % ,,

Mucor racemosus has produced :—

According to	After	In	At	Alcohol
FITZ (IX.) . .	"many months"	must	25–26° C.	3.5–4.0% by weight
BREFELD (XIII.)	...	nutrient soln.	15° C.	4.5% ,,
BREFELD (XIII.)	...	...	high temp.	up to 5.5% ,,
PASTEUR (III.) .	2 years	wort	room temp.	3.3% ,,
PASTEUR (III.) .	2½ years	...	...	3.4% ,,
FITZ (XI.) . .	1½ months	must	25–30° C.	2.3–2.7% ,,
HANSEN (VIII.) .	12 months	wort	room temp.	7.0% by volume

According to U. GAYON (V.) the production of alcohol by *Mucor spinosus* ceases as soon as the amount formed attains 1.5 to 2.0 per cent. ; but Hansen, on the other hand, succeeded in detecting 5.4 per cent. by volume in wort cultures at 22° C., after 6½ months. *Mucor circinelloides*, according to GAYON (VI.), produces 5.5 per cent. of alcohol. *Mucor erectus*, according to Hansen, when grown in wort cultures at room temperature, furnishes 8 per cent. by volume in 2½ months, but only 7 per cent. at 25° C. It therefore probably forms an exception to the rule applying to all the other *Mucoreæ* mentioned, namely, that they produce larger quantities of alcohol at higher temperatures than at lower. Thus, according to FITZ (IX.), *Mucor racemosus* is very sluggish

in this respect at temperatures below 15° C. The weakest of all seems to be *Rhizopus nigricans*, which, according to Brefeld (XV.), ceases its activity as soon as 1.5 per cent. of alcohol has been formed. None of these species of Mucor can be used for distillery purposes, since they are far surpassed in competition by a number of true yeasts, which are capable of producing an equal quantity of alcohol in a much shorter time, and also to carry on the fermentation much further. With respect to a few other species, proposed and employed for this purpose, further particulars will be found in §§ 240 and 242.

Carbon dioxide, being the most important, in quantity, of the other products of intramolecular respiration in *Mucoreæ*, will be dealt with next. The reliable quantitative determinations of the production of this gas are very few in number, and for these we are mainly indebted to Fitz (IX.) and Gayon (VI.). The former worker, as the mean of nine experiments with *Mucor racemosus*, determined the ratio (by weight) of alcohol to carbon dioxide as 100 : 123.1 ; and Gayon obtained similar values for *Mucor circinelloides*, whereas, according to the Gay-Lussac equation, the ratio should be 100 : 95.6. The formation of aldehyde and succinic acid in this process was first observed in cultures of *Mucor racemosus* by Fitz, and in those of *M. circinelloides* by Gayon, the last-named estimating the amount of this acid as 2.07 grms., and the amount of the resulting glycerin as 0.92 grm., per 100 grms. of saccharose consumed. These experiments with *Mucor racemosus* were afterwards resumed, with cultures of reliable purity, by O. Emmerling (III.), who obtained the following results from a culture grown for three weeks in a nutrient solution of saccharose and mineral salts, at 25° C., air being excluded : alcohol, 1.46 per cent. ; succinic acid, 0·02 per cent. ; glycerin, 0.12 per cent. The mutual ratio between these three products is 100 : 1.4 : 8.3. The amount of succinic acid seems to differ with different species ; at least Brefeld (XIII.) states, on the basis of his experiments with *Rhizopus nigricans*, *Mucor mucedo*, and *M. racemosus*, that cultures of the first of these organisms yield the relatively largest proportion of acid, and those of the third the smallest, though still more than is furnished by beer yeast under similar conditions. These results presumably relate solely to succinic acid ; at least this is probably the case with *M. racemosus*, since, according to Ph. Biourge (I.), this organism does not produce any volatile acids. Products of the ester class have been often observed to result from the intramolecular respiration of *Mucoreæ ; e.g.* an odour resembling that of pears, in cultures of *M. racemosus* by Brefeld, and one recalling that of plums, in *Mucor circinelloides* by Gayon.

We will now inquire as to the **kinds of carbohydrates** which may be drawn upon by the *Mucoreæ* for the purpose of forming

alcohol; and we will divide these into sugars on the one hand and starch and dextrin on the other, so as to be able to classify the species of this genus on the basis of their practical applicability. Starch and dextrin do not yield alcohol direct, but only after they have been converted into sugar; consequently they can only come into consideration in the case of such *Mucors* as are capable of producing saccharifying enzymes. These species will be fully described in the next chapter, and for the present we will deal solely with the formation of alcohol from sugars, and with the inversion of the latter. So far as is known, **saccharose** is split up by only two of the species hitherto mentioned. One of these—according to Fitz (IX. and XI.), Brefeld (XV.), and Hansen (VIII.)—is *Mucor racemosus;* the other was observed by the last-named worker, and is probably nothing more than a sport of the same organism. These two species produce an inverting enzyme, which faculty constitutes a sharply defined distinction between them and the *Mucor* and *Rhizopus* species hitherto known. Hansen's comparative experiments revealed the importance of this physiological characteristic as a means of differentiating the individual species; and its value for the purpose in view is so much the greater from the fact that its occurrence in these two species, and absence in all the rest, is quite independent of the environment; which is more than can be said of morphological characteristics. It is therefore advisable to point out at once that saccharose cannot be inverted or fermented by *Mucor mucedo*, *M. erectus*, *M. spinosus*, *M. alternans*, *M. circinelloides*, *M. Rouxii*, or *Rhizopus nigricans*, though they can draw upon it as a source of carbon. On the other hand, with the exception of *Rhizopus nigricans*, they can all attack **maltose** and convert it into alcohol. Whether the fermentation is preceded by a fission of this disaccharide into hexoses, as occurs in fermentation with true yeasts, has not yet been investigated. None of them is capable of converting **galactose** into alcohol direct, though they can do so after inversion, as shown by Fitz (XI.) in the case of *Mucor racemosus*. This latter is therefore equally incapable with the other six species of producing an enzyme that can hydrolyse milk sugar. According to Dubourg (I.), **trehalose** can be fermented to alcohol by *Mucor alternans*, though **raffinose** cannot. From **dextrose** and **lævulose** each of the above-mentioned *Mucoreæ* can produce alcohol by intramolecular respiration; and the same naturally also applies to the **invert sugar** formed from saccharose, by chemical reagents or the preparatory activity of other organisms. Presuming the above-mentioned preliminary hydrolysis of maltose to be correct, the formation of alcohol by the intramolecular respiration of *Mucoreæ* will fall under the same law as has already been found to prevail, without exception, in the alcoholic fermentation excited by *Saccharomycetes*

and other budding fungi, namely, that the hexoses alone are capable of furnishing alcohol direct.

Basing on the one hand on the impregnability of saccharose, and on the susceptibility to dissociation of the hexoses on the other, GAYON (IV.) has attempted to elaborate a process for extracting sugar from molasses. The chief feature of the process is the decomposition, by *Mucor circinelloides*, of the invert sugar, which, as is well known, prevents the crystallisation of the accompanying saccharose in molasses; the resulting alcohol is then distilled off, and the saccharose is finally separated from the residue by crystallisation.

In the manufacture of snuff, a certain part seems to be played by the intramolecular respiration of *Mucors*, especially in connection with the after-fermentation in the so-called "pig-tails" to which reference has already been made in § 105. Here, as also in tobacco pressed into casks, MICIOL (I.) very often found *Mucor mucedo*, in addition to the *Sporodinia grandis* at that time known as *Mucor flavidus*. J. BEHRENS (III.) afterwards found a second species (*Mucor racemosus*) both in the pig-tails and on tobacco leaves, both green and dry; and he also observed that this latter organism acts therein as a producer of alcohol, drawing upon the sugar contained in the "sauces" (§ 105). On the other hand, he rarely succeeded in finding Mucor species on tobaccos that were not treated with "sauce."

With regard to the part played by Mucors in turning bread mouldy and in the rotting of fruit, this and correlative questions will be dealt with in the final section.

O. JOHAN-OLSEN (II.) in describing the preparation and composition of Gammelost (a Norwegian cheese), states that certain species of *Mucor*—*Mucor casei*, and *Chlamydomucor casei*, both new species—take part in the ripening of this product. The experiments of WEIGMANN (X.) confirmed that, owing to their strongly developed capacity for degrading casein, fungi of this kind are able to take part in the ripening of cheese, and also to affect the flavour of the article by forming pungent fission products.

CHAPTER XLV.

THE USE OF MUCOREÆ IN THE SPIRIT INDUSTRY.

§ 240.—Mucor Rouxii and other species of Amylomycetes.

The faculty of producing **diastatic enzymes** and thereby exerting a saccharifying action on both starch and dextrin, is possessed by a considerable number of the Mucors. For the earliest information on this point we are indebted to U. Gayon and E. Dubourg (II.), who observed this faculty in *Mucor circinelloides* in 1886, and also found that this organism was only capable of exerting the influence specified when it developed in aggregations of gemmæ, but not when in the form of its normal mycelium. Shortly afterwards they reported (III.) on further similar experiments with *Mucor alternans*, and demonstrated that, under equal conditions, this organism converts starch into maltose and not into a hexose. The faculty is lacking in other species, *e.g.* *Mucor racemosus*, which, according to Fitz (IX.) also leaves inulin unacted upon. There are other more powerful species of Mucors, which, on account of their practical importance for the spirit industry, will now be fully described.

For the preparation of rice spirit there is produced in China, Cochin China, and the neighbouring countries, an article known as **Chinese Yeast**, and put on the market in the form of flat mealy balls, about the size of a half-crown. Its preparation, composition, and application were first described in 1892 by E. Calmette (I.), whose reports were extended and supplemented by C. Eijkman (II.) in 1894. According to these authorities this so-called yeast is prepared by mixing equal quantities of husked rice—steeped in cold water—or rice meal and water, and pulverised aromatic drugs. The number of the latter varies, in different recipes, up to 46, but always includes garlic and galanga root. The resulting stiff paste is divided into small cakes of the shape mentioned above. These are laid on mats, previously coated with a thin but close layer of moistened rice husks (paddy), or are covered over with a layer of rice straw, and are kept in the dark for two to three days, at an air temperature of about 30° C. The balls, which by this time smell like yeast and have become coated with a fine

white velvety covering, are next dried, either by exposure to the sun, or (in rainy weather) by gentle fire heat, and are then packed into bags for sale to the distillers. In India and China these balls are employed in the following manner: about 1½ parts by weight of the finely crushed or ground mass are sprinkled over 100 parts of husked rice, that has previously been softened by boiling in water and cooled down sufficiently by spreading it out on mats. The two are intimately mixed together, placed in earthenware pots holding about 4½ galls. (20 litres), so as to half fill the same, and are left covered up. The starch gradually saccharifies, and the mass consequently becomes partly liquid. At the end of about three days the pots are filled up with river water, and the sweet liquid is left to undergo alcoholic fermentation, which sets in very soon. This stage lasts two days, and the mash is then distilled in leaden retorts heated by direct fire. Each 100 kilos. of rice furnish about 60 litres of spirits, containing about 36 per cent. of alcohol.

The **flora of Chinese yeast** consists of bacteria, which, however, are of no moment; then yeast cells, which must be regarded as the exciting agents of the alcoholic fermentation; and certain Mucors, which effect the saccharification of the starch. Of the last-named organisms, which alone concern us here, Calmette isolated a species, which, in honour of his teacher and colleague, E. Roux, he named *Amylomyces Rouxii*. He did not, however, ascertain anything definite respecting the morphology of this fungus and its position in the botanical system; but it was afterwards recognised as a Mucor by Eijkman, and its name changed to *Mucor amylomyces Rouxii*. The first thoroughgoing investigations regarding the progress of development of this organism were published by C. Wehmer (XI.) in 1900, who at the same time proposed to shorten the name to *Mucor Rouxii*. According to this worker the sporangiophore—which is about 1 m.m. long and 7 to 14 μ broad—is generally branched, and in many cases is decidedly sympodial (see Fig. 118). According to the conditions of nutrition the sporangia are either upright or declinate, globular, glass-clear and yellowish when ripe, and attain up to 50 μ in diameter. When they fall into decay a considerable remainder of the smooth, colourless membrane is left as a collar on the sporangiophore. The columella (Fig. 119) is globular, slightly flattened, smooth, colourless, and measures 23 to 32 μ by 20 to 28 μ. The spores are colourless, glabrous, and generally elongated (5 to 2.8 μ), rarely globular, and completely fill the sporangium. It is worthy of mention that the failure to form sporangia noticeable in many Phycomycetes is also observed here as a frequent occurrence, the sporangium, instead of ripening, either ceasing to develop more than half-way, or else germinating as a tube in place of forming spores. This phenomenon does not appear in cultures on rice, but most usually

occurs in those on sugar-agar, thus affording here a new and beautiful example of the influence of the conditions of nutrition on development. The mycelium produces an abundance of gemmæ, of different sizes and shapes, either globular (from 10 to 100 μ in diameter) or oval. They are colourless, or pale yellow to light brown, and have a very thick, smooth, colourless membrane. It is solely in the form of these resting cells that the fungus appears in Chinese yeast, to which, as Calmette has shown, it gains access from the rice husks and rice straw. This explains why, in many places, the manufacturers of Chinese yeast consider it indispensable to press a few moistened rice husks into each of the fresh, pasty yeast cakes. As soon as the

Fig. 118.—Mucor Rouxii.

Sympodial branched sporangiophore, from a 7-days old culture on sugar-agar. Magn. about 160. (*After Wehmer.*)

Fig. 119.—Mucor Rouxii.

Sporangium almost entirely empty. Part of the wall still hanging in large shreds on the sporangiophore. In front of the globular columella are still lying three spores. From a culture on rice. Magn. about 230. (*After Wehmer.*)

gemmæ arrive in a suitable nutrient medium they put forth germinating tubes and develop into mycelia. Up to the present no zygospores or yeast-like budding cells have been observed in this fungus. When grown on solid media (steamed rice in particular) the mycelium gradually assumes a highly characteristic orange colour due to golden yellow drops (oil ?) appearing in the cell, but only at room temperature, though not at 40° C. The optimum temperature for the development of the mycelium lies between 35° and 40° C. According to Calmette, a temperature of 75° C. kills the organism within half-an-hour, and 80° C. is fatal in 15 minutes.

The same observer states that the diastatic enzyme produced by *Mucor Rouxii* exerts its most powerful effects at 35 to 38° C., and is destroyed on being warmed to 72° C. According to Boidin and Rolants (I.), the sugar thus formed from the starch is glucose. Like lævulose, saccharose, maltose, and lactose, this

sugar serves as a source of carbon to the fungus, and is burnt off provided there is direct access of atmospheric oxygen to the mycelium. If, on the other hand, the latter is compelled to remain submerged in the nutrient substratum, the glucose is also converted into alcohol. A. Sitnikoff and W. Rommel (I.) state that the same also occurs in presence of *d*-mannose, fructose, galactose, trehalose, maltose, dextrin, and *a*-methyl glucoside, but not in presence of raffinose, lactose, saccharose, melibiose, xylose, arabinose, rhamnose, tagatose, *β*-methyl glucoside, inulin, &c. The inability of this fungus to produce invertin was recognised by J. Sanguinetti (I.). This worker also performed a series of comparative experiments on the effective force and conditions of the diastatic enzymes produced by *Mucor Rouxii*, *M. alternans*, and *Aspergillus oryzæ*, and found that the last-named is weaker than the other two, so far as regards the saccharification of dextrin, the greatest power in this respect being exhibited by the second. The appearance of free acids in the cultures of *Mucor Rouxii* is also recorded by the same observer; Calmette supposes oxalic acid, Eijkman, on the contrary, lactic acid. The amount of acid may be so abundant as to kill the culture. The industrial utilisation of this fungus in Europe will be dealt with in the next paragraph but one. At present we will conclude with the remark that Boidin and Rolants' proposal to employ this fungus for converting into sugar, or alcohol, the dextrin left in the residues from distilleries operated on existing lines, would probably be too expensive outside the laboratory.

β-Amylomyces and *γ-Amylomyces* are the names of two species of *Mucor* which have recently found employment in the so-called **amylo process** (§ 242), instead of *Mucor Rouxii* (*a-Amylomyces*). Colette and Boidin found the *β* species on Japanese rice, the other (*γ-Amylomyces*) on Tonkin rice; and we are indebted to A. Sitnikoff and W. Rommel (I.) for fuller researches on their morphology and physiology. According to these workers, cultures of these two species on a nutrient solution containing glucose can be readily distinguished from those of *Mucor Rouxii*, by the circumstance that, whereas the latter develops as a weakly, barren mycelium almost entirely within the liquid, the *β* and *γ* species, on the other hand, also grow on the surface, forming well-developed aerial mycelia, which project above the level of the liquid a distance of as much as 3 c.m., and bring forth sporangia. The latter often develop in a special manner. Instead of the tip of the sporangiophore gradually enlarging to a sporangium, the swelling here constitutes merely a preliminary stage. It does not itself develop into a sporangium, but puts forth one or more threadlike processes which may then become crowned with sporangia (one each). This is illustrated in Figs. 120 and 121. In both species the ripe sporangia are

black, and of slightly flattened globular form, the transverse diameter varying between 45 and 95 μ, whilst the longitudinal diameter is about 15 μ smaller. The spores are oval or elliptical, brown in colour, and attain the following dimensions, in the

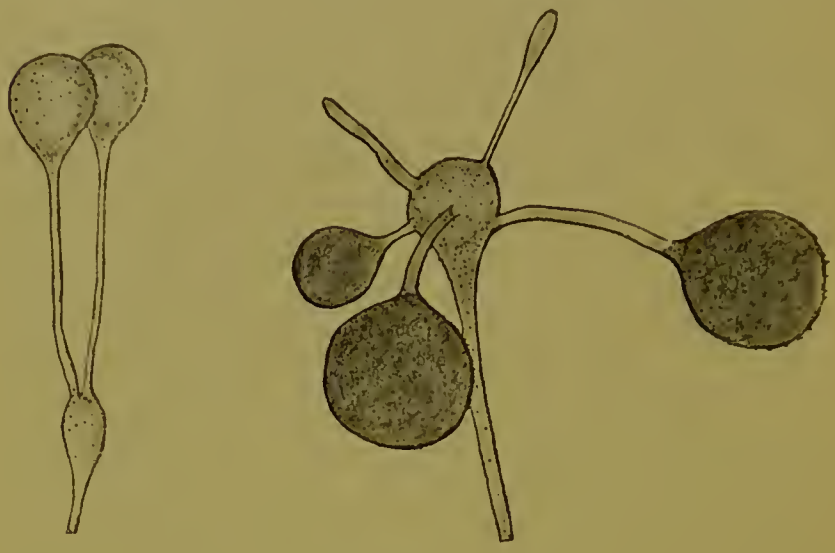


FIG. 120.—Sporangiation in β-Amylomyces.

The globular swelling at the apex of the hypha produces in the one case two branches, each carrying a sporangium, in the other case five branches, three of them exhibiting each a single sporangium in different stages of development. Magn. about 120. (*After Rommel's original drawing.*)

FIG. 121.—Sporangiation in γ-Amylomyces.

In the one example the knoblike swelling has put forth three branches, each with a sporangium; in the other, one of the branches is short and has no sporangium, the other two being long, declinate, and carrying sporangia. Magn. about 80. (*After Rommel's original drawing.*)

dry state: β-species, length about 9 μ, breadth about 5.7 μ; γ-species, 7 and 4 μ respectively. The formation of gemmæ on the mycelia is of very frequent occurrence, especially when air is excluded. The behaviour of these two fungi towards

sugars was examined by the Linduer micro-fermentation method. The results showed that glucose, fructose, *d*-mannose, galactose, maltose, and dextrin are fermented by both these species, alcohol being formed; whilst, on the other hand, saccharose, melibiose, raffinose, and inulin are fermented by the *β*-species solely, and trehalose by the *γ*-species exclusively. The *a*- and the *β*-methyl glucoside remained unacted upon by either. Potato-starch paste, liquefied by an addition of nutrient salts, was saccharified more effectually by each of these two species than by *Mucor Rouxii* in a comparative experiment. This activity has already been utilised in practice, and preference is given to the two new species in working the amylo process. In respect of energy in the production of alcohol they appear to be inferior to the other; but this does not lower their efficiency when used merely as saccharifying agents.

Mucor Cambodja, which was isolated from Chinese yeast and carefully examined by T. Chrzaszcz (I.), will produce 1·06 per cent. of alcohol in twenty days in a nutrient solution containing 10 per cent. of dextrose. Sporangiation proceeds in a similar manner to the two last-named species of Amylomyces; but the organism differs from these by forming rhizoid processes, and therefore presumably belongs to the genus *Rhizopus*.

§ 241.—Ragi and Tapej.

The raw material employed in Java for the production of arrack consists of waste molasses from sugar works, containing about 20 to 30 per cent. of glucose (or invert sugar) and 25 to 35 per cent. of saccharose. This molasses is reduced to about 15° Bé. (26·6° Ball.) by dilution with river water, and is then treated, in order to reliably accelerate alcoholic fermentation, with an auxiliary, which is comparable with the leaven (§ 148) used in European distilleries, and is known as **Tapej**. This is prepared from rice by the aid of a second auxiliary material, which the Malay natives of Java term **Ragi** or **Raggi**, and the Chinese settlers call **Peh-Khak**.

According to A. G. Vorderman (I.) this Ragi is prepared by crushing together pieces (rich in sugar) of the sugar-cane and the rootstock of galanga (*Alpinia galanga*), and making the product into a paste with rice meal. Went and Prinsen Geerligs (I.) state that garlic and other aromatic plants also form part of the mixture. The paste is divided into strips, which are dried in the sun, then broken up, mixed with water and lemon juice, and left to stand for three days. The liquid portion is afterwards poured off, the coarser vegetable fragments are removed, and the pulpy residue is made up into flat, round cakes, about 4 c.m. in diameter, which are left to dry and harden in the sun. Generally, however, they must first be laid in rice straw for a couple of days, or

else a few fragments of fresh rice straw are kneaded in with the cakes. The dried Ragi cakes—which are sometimes called **Java yeast**—form an article of commerce in Java.

According to EIJKMAN (II.), Tapej, or, as it is also called, **Tsao**, is prepared by the aid of Ragi, by boiling husked Mochigome rice (*Oryza glutinosa*, known as "Ketan" in Java) in water till soft, spreading it out in a thin layer till cool, sprinkling it over with powdered Ragi (with which it is well mixed), and packing the whole into a cylindrical vessel (with a perforated bottom), which is then covered up with a mat and left alone for two days. At the end of this time the rice will be found changed into a semi-fluid, sour-sweet, coherent mass, which is now called Tapej. As already mentioned, it is used to excite fermentation in molasses. Its application will be again referred to in the next section.

The **flora** of Ragi, and of Tapej as well, comprises three groups of micro-organisms: first, bacteria, which need not be considered here; secondly, budding fungi, which will be dealt with in a later section; thirdly, fungi belonging to the family *Mucoraceæ*. The latter alone are concerned in the saccharification of the starch in Tapej, and with the liquefaction of the mass. WENT and GEERLIGS (I.) discovered two forms of these in Ragi, and bestowed on them the names *Chlamydomucor oryzæ* and *Rhizopus oryzæ* (§ 237), but left open the question as to the probability of both forms belonging to one and the same species. One of them is in many respects similar to the *Amylomyces* (*Mucor*) *Rouxii* already described, and, like this latter, also has its habitat on rice straw. Nevertheless, certain differences have been observed between them, especially with regard to the absence of the faculty (possessed by the *Mucor* aforesaid) of producing alcohol from sugar when oxygen is excluded. In Ragi this species is found exclusively in the form of gemmæ, which are rich in glycogen, strongly refractive of light, and, according to C. WEHMER (IX.), retain their power of germination for more than one and a half years when in the dry state. In Tapej they then germinate to a mycelium, which, however, rarely (and then only sparingly) succeeds in forming the (black) sporangia in that medium, though it will do so abundantly in pure cultures on boiled rice, &c. A certain interest attaches to the physiology of *Chlamydomucor oryzæ*. By the action of its diastatic enzyme it forms dextrose from starch. The amount of this sugar formed, however, depends on the kind of starch employed, the results obtained by Went and Prinsen Geerligs being: with Indian meal and potato flour, 8 per cent.; arrowroot, 16 per cent.; wheaten flour, 29 per cent.; ordinary rice, 44 per cent.; and Mochigome rice, 64 per cent. As is well known, the starch granules of this last-named seed consist mainly of amylodextrin,

accompanied by a little erythrodextrin. It would therefore seem as though the fungus in question is only capable—preferably or perhaps exclusively—of saccharifying this carbohydrate, and not the granulose which forms the chief constituent of starch granules in the other kinds of meal specified. Hence this fungus might possibly be employed as an auxiliary in roughly estimating the percentage of granulose in starch. The yield of sugar obtainable by the action of this fungus on starch is somewhat low. Thus, for example, in an experiment recorded by PRINSEN GEERLIGS (II.), 100 parts of amylodextrin furnished: 110 parts of dextrose by saccharification under pressure with an acid, but only 68.5 of dextrose when saccharified by the fungus. The latter consumes a certain amount of the sugar as a source of carbon for its own needs; and it can also utilise alcohol for the same purpose. The nitrogen requisite for building up the cell it can take up from peptone, asparagin, and ammonium salts, but not from nitrates and nitrites. It is incapable of producing invertin.

A new species, allied to the foregoing, has been described, under the name *Mucor javanicus*, by C. WEHMER (XIII.). It occurs both in Ragi and Chinese yeast, mainly in the form of gemmæ, which retain their germinative power for at least five years in these media. Morphologically, they closely resemble *Mucor alternans* and *M. circinelloides*. The mycelial hyphæ measure 12 to 15 μ in thickness. With increasing age the presence of oil drops in the plasma imparts to the latter a decided coloration, which, however, is not so powerful as in the case of *Mucor Rouxii*. Similar fatty inclusions are also found in the gemmæ. These latter are put forth by the mycelium, both upon and in the nutrient substratum, and attain a diameter of 4 to 20 μ, being therefore far smaller than those of the last-named species. When the nutrient substratum is sufficiently consistent, there springs up from the mycelium a dense herbage of sporangiophores, which form sympodial branches, attain a height of 1 c.m. and over, and develop a globular sporangium on each of their six or more branches. The dimensions of the sporangium increase with the height of the support and vary between the limits of 50 and 20 μ in diameter. The colour of the sporangium is yellowish-grey to brownish; and the membrane is almost always smooth, as is also the globular (10 to 35 μ) columella. The spores are globular to elliptical, colourless, smooth and thin in the membrane, and measure 5 to 6 μ, or 5 to 7 μ by 4 to 5 μ. Up to the present, no zygospores have been observed. The optimum temperature of development lies between 35 and 40° C. When developed in saccharified malt extract or in a solution of dextrose and nutrient salts, this fungus produces an appreciable amount of alcohol; neither this matter, nor the

capacity of the fungus for saccharifying starch, has, however, yet been thoroughly investigated.

Until further particulars are forthcoming, we must regard *Chlamydomucor oryzæ* as the principal agent in the diastatic process that goes on in the incipient Tapej. The gradually increasing quantity of sugar resulting from the activity of this fungus then furnishes the desired nutrient material for yeasts, which are either already present in the Ragi or have found their way—through the water or from the air—into the Tapej, where they reproduce freely and thus impart to this material the property forming the object of its preparation: namely, to quickly excite fermentation in the dilute molasses into which it is introduced when ripe. Further particulars respecting the yeasts in Tapej and the application of this material will be given in a later section.

The natives of Java also prepare Tapej for another purpose. They express the sweet juice, allow it to thicken in the sun, and pack it in small twists made of Pisang leaves. Here it crystallises into crumbling masses, which are called Brem and furnish a favourite sweetmeat. The composition was ascertained to be in one case: dextrose, 69 per cent.; dextrin, 10.6 per cent.; ash, 1.2 per cent.; and water, 18.7 per cent. In addition, Tapej is also eaten alone by the natives.

§ 242.—The so-called Amylomyces Process.

The **Amylomyces process** (or **Amylo process** for short) is the name given to the process for the industrial utilisation of the diastatic activity of *Mucor Rouxii* and several allied fungi. A company, the "Société d'Amylo," was founded by A. Collette and A. Boidin (I.), who also, in 1897, took out in the name of this company a German patent for a "process for producing alcohol from starchy materials, by means of aseptic saccharification and fermentation with *Mucedineæ*, characterised by the feature that, in order to prevent the combustion of starch during aseptic saccharification by means of *Mucedineæ*, the sterilised raw material, treated with water, is subjected to the passage of a current of germ-free air, the material being meanwhile advantageously kept stirred by mechanical means; after which the air supply is cut off and the material is pitched with yeast, which, by means of the consequent rapid liberation of carbon dioxide, prevents the combustion of the alcohol already formed by the *Mucedineæ*."

We are indebted to A. Fernbach (II.) for a lucid description of the practical performance of this process in the patentees' works, the maize distillery at Seclin near Lille. Of this description only the main points can be reproduced here. The maize, mixed with twice its own weight of water, is steamed for

three hours, under a pressure of 3½ to 4 atmospheres, in a Henze high-pressure steamer, and is then discharged into a preliminary mashing tun previously charged with 1 part, by weight, of green malt for every 100 parts of maize, and sufficient cold water to reduce the temperature of the whole to 70° C. Presumably the sole purpose of the added malt is to liquefy the swollen starch of the steamed maize, and thus render it more readily accessible to the saccharifying action of the fungus that is afterwards added. After an hour's stirring the mixture is transferred to an autoclave, where it is sterilised at 120° C., and whence it is forced, by pressure, into a cylindrical metallic fermenting tun, which is enclosed on all sides. In construction, this vessel resembles the apparatus used in breweries and distilleries for the production of pure-culture yeast, and therefore the intrusion of all extraneous germs is excluded with certainty. To sterilise the interior, a portion only of the sterilised wash is introduced at first and is treated with sulphuric acid, being afterwards boiled a short time by direct steam. By this means any germs that may be adhering to the upper parts of the inside are loosened by the condensed steam, and swilled down into the hot, acid mash, where they are quickly killed. The acid is said to be sufficiently neutralised by the lime present in the subsequently introduced remainder of the mash, which is delivered from the autoclave under pressure. The total capacity of the fermenting vessel, as used at Seelin, is about 23,800 galls. (1080 hectolitres), and the charge measures about 22,000 galls. (1000 hl.). At the present time this charge is prepared from about 18 tons (18,000 kilos.) of material, instead of 10 tons in the earlier days of the process. As soon as the fermenting vessel is charged, the supply of steam is shut off, and sterilised air is then introduced, under pressure, through a special pipe, so as to keep the mash under pressure during the commencement of cooling, which soon begins. The cooling is effected by the aid of water, which is caused to trickle down the outside of the fermenting vessel from a perforated tube surrounding same. The escaping air (as also the carbon dioxide given off during fermentation) is removed through a pipe dipping into a water seal, to prevent direct connection with the atmosphere. By the end of five hours the temperature of the contents will have sunk to 38° C. They are then inoculated with a pure culture of the *Amylomyces*, rich in spores, contained in a 3-litre Pasteur flask charged with 100 c.c. of beer-wort and 100 c.c. of boiled rice; this leaven is introduced through a previously closed special pipe in the upper part of the fermenting vessel, which pipe is immediately recapped. The internal stirring apparatus, the shaft of which passes through a germ-tight stuffing box, is next set in motion, and during the succeeding twenty-four hours a gentle current of air is passed through the mash, which at the same

time is kept in motion to prevent the development of a mycelial herbage on the surface of the mash, since this would lead to a loss of material by brisk respiration.

In twenty-four hours after inoculation, the entire mash is permeated with the mycelium of the *Amylomyces*. The formation of sugar now proceeds with rapidity. Its fermentation, however, according to the patent in question, is not (or at most not to any considerable extent) effected by *Amylomycetes* capable of exercising this function, but rather by a suitable pure yeast, which at this stage is added to the mash cooled to 33° C., the amount taken being about 5 grams grown in about ½ a litre of nutrient solution in a Pasteur flask. The reproduction of this comparatively small sowing proceeds rapidly during the next twenty-four hours, thanks to the uninterrupted admission of air. The latter is then excluded, whereupon both species of fungi act conjointly during the three following days: the *Amylomyces* saccharifying the hitherto unconverted portions of starch and dextrin, whilst the fermentation of the sugar already present and that continually forming, is chiefly effected by the yeast. The principal task assigned to the yeast by the patentees is not this fermentation, but merely the formation of carbon dioxide, thereby ensuring the presence of an oxygen-free atmosphere within the fermenting vessel, and thus precluding the consumption of the alcohol by the *Amylomyces*. Collette and Boidin (II.) afterwards, in 1898, took out an additional patent, in which it is explained that the yeast may be entirely dispensed with, if the saccharified mash be freed from oxygen by stopping the supply of air immediately the iodine test reveals that the conversion of starch is completed; then passing carbon dioxide through the mash for an hour, and afterwards leaving the *Amylomyces* to proceed with its task alone. It is nevertheless a fact that yeast is still added in the Amylomyces process plant erected in Austria-Hungary since the date of this additional patent. An English patent for a mechanico-technological modification of the process was also taken out by Collette and Boidin (III.) in 1898.

An important simplification of this process is practised in the Anker Distillery at Antwerp, as reported by O. Saare (I.). In this case the sterilisation in the autoclave and the boiling with sulphuric acid are omitted. The mash, prepared from steamed maize with an addition of 2 per cent. of malt, is transferred direct to the fermenting vessel, where it is boiled for a short time by steam under ordinary pressure, then cooled and aerated, the inoculation with the *Amylomyces* culture being afterwards effected in the manner already recorded. When this has done its work and the mash no longer reacts with iodine, an addition of yeast is given, the kind used being the No. II. race of the Berlin Station (§ 245).

It is not the author's province now to pass judgment on the value of the Amylomyces process from a technological and economic standpoint, the more so because the reader interested in this matter will find more precise data in the review published by M. DELBRUECK (III.). The chief advantage of the Amylomyces process is the abolition of the expensive additions of malt requisite in the older method of saccharification, the amount formerly needed being up to 15 per cent. in the case of maize, and 2 to 3 per cent. in the case of potatoes. With regard to the yield furnished by the Amylomyces process, it is stated that in the Seclin works, 37.8 litres of absolute alcohol are obtained per 100 kilos. of maize containing 57.5 per cent. of starch, a yield corresponding to 66.2 litres per 100 kilos. of starch. Owing to the large proportion of mycelial hyphæ, the residue filters easily, a circumstance of considerable value in French distilleries where the residues are not consumed on the premises for fodder, but are dried and pressed for further treatment.

Viewed from the mycological standpoint, the advantage, and at the same time a source of weakness, in this method lies in the inoculation with such a small amount of yeast, and therefore in the abolition of the preparation and employment of a yeast-mash (§ 148). In the present case the mash is deprived of the bactericidal lactic acid, and is therefore readily infested by injurious germs, should the latter be present at the start, or even find their way in as a result of carelessness in the act of inoculation, at a time when only a few individual organisms of the *Amylomyces* or yeast are present. On the other hand, it is claimed in favour of the raw spirit formed in this process that it is very low in fusel oils. Another question (of a purely economic character) is how far the extra output of alcohol is counterbalanced by the increased outlay in fuel for sterilising the mash, and by the interest on and depreciation of the necessary plant, which latter very soon wears out.

Finally, it should also be mentioned that, since 1898, the aforesaid patentees have replaced *Mucor* (*Amylomyces*) *Rouxii* by another species, namely, the so-called *β-Amylomyces*, or *Mucor β*, described in § 240. This organism is capable of dealing with more highly concentrated mashes than the other, and enables a charge of 25,000 kilos. of maize to be mashed to 1000 hl. (22,000 galls.) of goods. According to Boidin, a third species—namely, that already mentioned as *γ-Amylomyces*, or *Mucor γ*—is able to carry the fermentation 0.1 to 0.2 Balling further than is attainable by using the others aforesaid. Latterly, however, this fungus seems to have been abandoned again, since it was reported, at the commencement of 1900, that a Luxemburg grain distillery, working with the Amylomyces process, had superseded the foregoing species of *Mucor* by *Aspergillus oryzæ* (for description of which see a later section),

and had by this means succeeded in producing 39 litres of alcohol per 100 kilos. of rye taken. It may also be mentioned that in this distillery the steaming, mashing, cooling, and fermentation were all carried out in one and the same vessel, specially constructed for the purpose. The aforesaid employment of this last-named fungus is also protected by a patent taken out by COLLETTE and BOIDIN (IV.).

These same workers (V.) have also recommended the use of *Mucor Rouxii* for raising dough, especially in hot countries whither pressed yeast cannot be shipped. With this object they prepare the fungus in question according to a special process patented by themselves.

SECTION XII.

FORM, STRUCTURE, AND CHEMICAL COMPOSITION OF THE YEAST CELL.

CHAPTER XLVI.

MORPHOLOGY AND LIFE-HISTORY OF THE YEASTS.

§ 243.—Position of the Saccharomycetes in the Botanical System.

It was stated in § 220 that only a few classes of the sub-kingdom of Mycomycetes exhibit the faculty of producing endogenous spores, namely, the Ascomycetes. Consequently the latter may be defined as endosporogenic Mycomycetes. In the same place it was also mentioned that the organ wherein the endogenous spores are formed in this class is not called a sporangium but an **ascus**, and differs from the sporangium both as regards the greater fixity of shape and also the number, shape, and method of formation of the spores therein contained. These spores are therefore called **ascospores**.

The fundamental characteristic distinguishing the ascus from the sporangium resides in the behaviour of the bearer of the inherited properties of the organism, namely, the cell nucleus, during sporulation. The sporangium (p. 11) is polynuclear from the outset, whereas the ascus contains only a single nucleus at the commencement of sporulation. The nucleus—as will be more fully described in § 250—reproduces by subdivision into as many daughter-nuclei as there will be ascospores. In the sporangium, on the other hand, as many nuclei as there will subsequently be endospores are transferred from the aseptate mycelium to the sporangium before the separating membrane is formed.

It is only in the least highly developed *Ascomycetes* that the ascus springs direct from the mycelium; in all the rest it is developed in or upon a special organ called the **carpoascus**, and all the Ascomycetes fructifying in this manner are grouped into the sub-class of *Carpoasceæ*. Antithetical to this class are the lower *Ascomycetes*, the *Gymnoasceæ*, the asci of which are naked; and it is to this latter group that the most of the technically important yeasts belong.

An examination of the development of the carpoasci of a number of species belonging to the first sub-class has revealed

that here the asci are produced by a special organ, to which the name **ascogone** has been given. This is a unicellular or polycellular organ springing from the mycelium and putting forth the asci either direct or upon branched processes. At the same time it surrounds itself with an envelope, of one or several layers, thus broadly completing the formation of the carpoascus. The shape of the envelope and therefore of the entire carpoascus, forms the principal characteristic on which the sub-class of the *Carpoasceæ* are allotted their position in the botanical system. When the envelope does not enclose the ascogone on all sides, but merely serves as a support therefor (or for the asci) on the basal side, leaving the others free, then a disc-like or cup-shaped fruit is formed, which is called an **apothecium**. *Ascomycetes* producing fruit of this kind are termed Discomycetes. If, on the other hand, the ascogones or asci remain surrounded on all sides by the envelope when ripe, then the fruit is termed a **perithecium**, *i.e.* globular, oval, or in the form of a short bottle; and the *Ascomycetes* producing same are classed along with the order of *Pyrenomycetes*, or *nuclear fungi*, the second of the two orders into which the sub-class of *Carpoasceæ* has been divided.

If the perithecium is of a kind that exhibits no aperture at all during the whole period of its existence, so that the contained asci or ascospores cannot escape into the open air unless the perithecial membrane breaks up or is destroyed, then the perithecium is termed **cleistocarpous** or enclosed. All the *Pyrenomycetes* bearing fruit of this character are included in the sub-order of *Perisporiaceæ*. The common greenish-blue bread mould belongs hereto.

On the other hand, when the perithecium is of such a type that, in the ripening process, it develops an orifice through which the asci or ascospores are able to escape, it is then said to be **peronocarpous**. The second sub-order of the *Pyrenomycetes* exhibits this kind of fruit, and is called *Sphæriaceæ*.

Scheme of subdivision of the Ascomycetes Class.

- **Ascomycetes** (Mycomycetes with endogenous spores)
  - (*a*) Asci naked . . . . . *Gymnoasceæ.*
  - (*b*) Asci with envelope (Carpoascus) *Carpoasceæ*
    - (*a*) Carpoascus is a perithecium: *Pyrenomycetes*
      - 1. Perithecium cleistocarpous . *Perisporiaceæ.*
      - 2. Perithecium peronocarpous . *Sphæriaceæ.*
    - (*β*) Carpoascus is an apothecium . . . . . *Discomycetes.*

Of the *Discomycetes*, only a single species, *Sclerotinia Fuckeliana*, is of any importance in connection with the scope of the present work. The conidial fructification of this organism produces "sweet-rot" in grapes, and on this account will be fully described in a separate chapter. Of the sub-order *Sphæriaceæ* we shall have three species to consider, namely: *Claviceps purpurea*, or the sclerotium of this organism, the detection of which in flour and bread is of interest to us; also *Sphærella Tulasnei*, and *Sphærella intermixta*. Of the sub-order *Perisporiaceæ* we shall have to make the acquaintance of a number of species belonging to the families *Penicilleæ* and *Aspergilleæ*. These *Carpoasceæ* will be dealt with in the penultimate section, wherein an example will be given of the structure and progressive development of the carpoascus.

FIG. 122.—Carlsberg Bottom-Yeast, No. 2.

Cells taken from a culture on wort-gelatine, and exhibiting the formation of 2 to 3 ascospores in each. Magn. 1000. (*After Hansen.*)

Now the *Gymnoasceæ* are characterised by the nakedness of the asci, which remain devoid of integument during their entire existence. This sub-class (or order) can be divided into three families. In the *Saccharomycetes*, which constitute the lowest family and therefore the most simply constructed of all *Ascomycetes*, the mycelium itself is converted into an ascus, and there is here no separation into vegetative and fructificative organs. An example of this is given in Fig. 122. A distinction between the organs is, however, exhibited in the other two families, the asci being articulated from the mycelium in one of them, namely, the *Exoasci*, where they form lateral offshoots resting on the mycelium. Finally, in the third family, the *Gymnoasci*, which forms the connecting link with the *Carpoasci*, incipient fructification is already found, as well as the intermediate organ, which we have already encountered under the name of ascogone.

We thus obtain the following scheme of subdivision of the Order Gymnoasceæ:—

Order, or Sub-class.			Family.
Gymnoasceæ (asci naked)	(*a*) Without ascogones	(*a*) Mycelial cells themselves become asci . .	1. Saccharomycetes.
		(*β*) Special branches of the mycelium develop into asci	2. Exoasci.
	(*b*) Form ascogones		3. Gymnoasci.

Of these three families of *Gymnoascea* we are, in the present book, concerned with only one, namely the *Saccharomycetes*, the *Gymnoasci* on the other hand being of no importance so far as we are concerned, whilst the *Exoasci* may be dismissed in a few words. The genera of this last-named family have been divided by R. SADEBECK (IV.) into two groups: the one comprising parasites, the other saprophytes. The action of one representa-

FIG. 123.—Endomyces decipiens Reess.

1. A mycelial branch, the lower part of which has developed three asci (*a*), each of them producing four hat-shaped ascospores; whereas the upper portion has separated into oidia (*b*). Magn. 320.
2. A fragment of mycelium entirely converted into oidia. Magn. 120.
3. A mycelial branch, which has formed oidia (*b*) at the top, but underneath has developed three chlamydospores (*c*). Magn. 240.
4. A mycelial branch with asci (*a*) solely. Magn. 320.
5. Pair of spores from such an ascus. Magn. 350. (*After Brefeld.*)

tive of the former group, namely *Taphrina Pruni* (also formerly known as *Exoascus Pruni*), will probably be familiar to the reader, viz. the malformation produced by this organism in green plums. According to SADEBECK (II.), the ascospores of this fungus, when grown in saccharine nutrient solutions, develop into a budding mycelium which excites weak alcoholic fermentation. A second species of this group, *Endomyces decipiens*, abounds in the lamellæ of the fruit of *Agaricus* (*Armillaria*)

melleus, a parasite attacking timber and well known to foresters as "honey fungus." This second species of the *Exoasci* is worthy of mention here; in the first place because, as can be seen from Fig. 123, it forms an excellent example of a pleomorphous fungus; chiefly, however, because the ascospores are similar in shape to those of *Saccharomyces anomalus* (to be described later on) and its congeners. It was on the basis of this similarity that, some years ago, and again more recently, more than one mycologist was led to discuss the possibility of the *Saccharomycetes* having originated (§ 244) from this species or one nearly allied thereto. However, no one has yet succeeded in arriving at anything beyond mere hypotheses in this connection; and moreover, similarly shaped spores are also found in other low *Ascomycetes*, e.g. *Ascoidea rubescens*. These few remarks are all that it is necessary to make, from our point of view, with regard to the *Exoasci* and *Gymnoasci*. On the other hand, all the following paragraphs, up to the penultimate section, will be concerned exclusively with the family Saccharomycetes.

FIG. 124.—Johannisberg Wine Yeast, No. 1. Young culture in wine must. Magn. 800. (*After Aderhold.*)

FIG. 125.—Schizosaccharomyces octosporus. One-day old culture in beer wort at 25° C. Magn. 1000. (*After Schiönning.*)

This family comprises three genera: *Monospora*, *Saccharomyces* and *Schizo-Saccharomyces*. As the name implies, the first genus is characterised by the fact that each ascus contains only a single spore. Up to the present only one species of this genus is known, namely *Monospora cuspidata* (discovered by Metschnikoff), which is parasitic on and fatal to Daphnidæ; it is, however, beyond our province. Henceforward we have merely to deal with the other two genera, *Saccharomyces* and *Schizo-Saccharomyces*. Of the latter, only a few species are as yet known. Some of these are, nevertheless, worthy of attention, either from their technical importance, or from a physiological standpoint, and will therefore be dealt with in a separate chapter as soon as our main task is accomplished, namely the study of the most important of the numerous species belonging to the genus *Saccharomyces*. In these latter the reproduction of the cells is effected by vegetative means; and under normal conditions almost exclusively by the process of budding described in § 219. An example is shown in Fig. 124. In the Schizo-Saccharomyces on the other hand—as will be more fully elucidated later on—this process retires into the background and gives place to another, which closely resembles the reproduction of bacteria by fission, as was described in § 42. In this case, instead of putting forth, like Saccharomyces, a bud which grows to the same shape and size as the mother-cell from which it sooner or later becomes detached, the cell develops a transverse partition wall, which then divides into two layers; and as soon as this is effected there is nothing to hinder the separation of the resulting daughter-cells (see Fig. 125). It is owing to this behaviour that the genus received the name Schizo-Saccharomyces.

The substance of the foregoing remarks can be employed for drawing up a scheme for subdividing the Saccharomycetes family:—

			Genus.
Saccharomycetes (Ascomycetes with naked asci, the mycelium itself acting as an ascus)	(*a*) The ascus develops only a single spore		1. Monospora.
	(*b*) Ascus Polysporous	(*α*) Vegetative reproduction by budding	2. Saccharomyces.
		(*β*) Vegetative reproduction by fission	3. Schizosaccharomyces.

Before entering upon the morphology and progress of development of the genus Saccharomyces, we will first occupy ourselves, in the following paragraphs, with the question of the alleged descent of the Saccharomycetes from other fungi; at

the same time the opportunity will be utilised for defining the limitations and mutual relation of the terms Saccharomyces, budding fungi, and yeast.

§ 244.—The Question of the Origin of the Saccharomycetes.

For some years after they had been recognised as living organisms (§ 12) the cells forming the chief constituent of beer yeast, wine yeast, and pressed yeast, had to be content with a position in a corner of the botanical system. Occurring as a rule in the unicellular form, or at most in aggregations of uniform members, they offered little inducement for the attention of the systematist. Meyen and Schwann bestowed on them the new generic name, *Saccharomyces* (§ 13), and relegated them to the fungoid kingdom. On the other hand, Kuetzing located them among the algæ, under the name *Cryptococcus*, for the very cogent reason that he failed to discover in them the formation of hyphæ, which is characteristic of the fungi. Although the views of the two first-named observers afterwards prevailed, and consequently the yeasts were recognised as belonging to the fungi, it was at first difficult to find suitable accommodation for them within the limits of this kingdom, no allied species being known with which they could be associated. In this emergency these and a few other similar fungi were classed together in a new group, namely that of the *Blastomycetes* (budding fungi), which was interpolated as an intermediate link between the sub-kingdom of the fission fungi on the one hand and that of all the other fungi on the other. The desire, which then soon arose, to find a connection between this outside group and an order of the sub-kingdom Eumycetes, was therefore not an artificial one, but in harmony with existing circumstances and fully justified. The manner, however, of effecting the gratification of this desire, and also the unscientific standpoint adopted as the basis of procedure, cannot be termed praiseworthy.

From the explanation given in the introduction to Vol. i. the reader is aware that the discovery of yeast as a living organism was coincident with a period when the dispute about primary origin raged very briskly. Now the yeast cell was one of the favourite objects of the attempt to prove the origin of organised from unorganised bodies. When, thanks to the labours of Pasteur and others, the theory in question was finally driven out of the realm of the exact sciences, many of its adherents considered an unconditional surrender was too much to be expected of them; and in order to preserve a little of the conception, they assumed that, even if the parentless origin

of the yeast cell from lifeless material could no longer be claimed with certainty, there was a probability of yeast being evolved *from* lower fungi on the one hand, and *into* higher fungi on the other.

Two circumstances favoured the inception of this opinion and prolonged its existence. One of them consisted in the lack of positively reliable pure-culture processes for enabling the observer to start with a single individual (in this case a single cell) and trace its development with uninterrupted supervision, to the exclusion of all other germs. It would be setting too small a value on the circumspection of the workers in question and the adherents of the assumption that yeast is descended from other fungi, were one to suggest, in their justification, that they were not even dimly aware of the indispensable character of such a condition. They must therefore have recognised that their method of working was unreliable and led to deceptive results, and must have been inwardly convinced that the solution of this problem was the next task to be attempted. We have to reproach these experimenters that they did not follow this inevitable knowledge to its conclusion, and did not devote their whole energy to first elaborating a truly reliable method of working. This we must do because the confusing results they conjured up by the aid of their officious culture methods, inflicted a twofold injury to science: first by aggravating the task of real research, and then by casting over the final results of the latter an anticipatory shadow of depreciation, which is solely due to these mycological necromancies.

The second circumstance favourable to the endeavours of those who tried to show the descent of yeasts from other fungi was the discovery of pleomorphism by Tulasne. Since the year 1851, this worker demonstrated, by a number of examples, that certain of the higher fungi were capable, under different conditions of nutrition, of assuming different forms; *e.g.* at one time appearing as a mycelial thread and putting forth conidia, at another as a sclerotium from which proceeded small pileated fungi. With such an instance we shall become acquainted, in the case of *Sclerotinia Fuckeliana*, later on. In fact, in other words, it was thereby proved that many of the living forms which had hitherto been regarded as separate species of fungi, merely formed a phase in the cycle of development of one and the same species of fungus. This theory of pleomorphism—which the inquiring reader will find fully described in the mycological handbooks already mentioned, more particularly in the treatise of A. Gilkinet (I.) recommended by A. de Bary—was established on an unassailable basis by its founder, by careful investigations of particular instances, and really marked a new epoch: not merely in mycology

alone. On the other hand, it did a good deal of mischief at first in the study of yeasts.

The obvious objection that the lack of reliable methods of pure culture could also militate against the certainty of Tulasne's results, can at once be disposed of by the fact that this authority worked with comparatively large fungi; and, moreover, a study of his works will very soon induce the conviction that such doubts are incapable of shaking the reliability of his discoveries. Furthermore, his determinations in connection with the progress of development—which were mainly based on microscopical researches—were afterwards confirmed by the cultures prepared by A. de Bary and others. The case was, however, different as soon as a crowd of imitators began attempts to prove the existence of similar pleomorphism in the less easily examined lower and lowest fungi. Whereas, in the case of the fungi examined by Tulasne—for instance a sclerotium of *Claviceps purpurea*—it was easy to select an individual and test its development, it became necessary, with the microscopically small fungi, to start with a multiplicity of individuals, *e.g.* a sample of yeast, owing to the absence of any method of pure culture. The detection of a small percentage of germs of other small fungi, *e.g.* a few conidia of a mould fungus, was not such an easy matter, though the same necessarily made their presence felt and even assumed a predominating position when the environment underwent a change in their favour. Either in ignorance of, or stubbornly misunderstanding, this state of affairs, Béchamp, in 1871, put forward the assumption that yeast cells could be developed from acetic bacteria. A year later, Trécul (II.) announced having made the same observation with spores of *Penicillium*. Conversely, according to Duval (I.), yeast cells were assumed capable of undergoing conversion into lactic acid bacteria. More extensive fallacies were advanced in 1875 by Robin (I.), giving renewed vitality to the reports of Pouchet (I.) and Bail, according to whom yeast cells are able to change to *Mucor*, *Penicillium*, *Aspergillus*, &c. Greater success was attained by H. Hoffman (III.), who even obtained a prize from the Paris Academy in 1870 for his conversions. For "staying power," however, the palm must be awarded to H. Hallier (II.), who still continued to uphold, in 1896, the hypothesis which had been relegated by A. de Bary to the "chronique scandaleuse" of science more than fifteen years earlier.

One extenuating circumstance to be considered in passing judgment on most of the above-named, is the fluctuations of meaning sustained by the term yeast in the course of time. A backward glance on this point is therefore necessary, even on this ground alone. Apart from the vague terminology which permitted (§ 13) every fermentative agent, even the fission fungi,

to be called yeast, sufficient indefiniteness attached even to the limitation of this term to the ferments belonging to the *Eumycetes*. For some years after Schwann's discovery, the sole botanical definition of yeast was: a unicellular organism, reproducing by means of a peculiar method (termed budding), and exhibiting the power of forming alcohol and carbon dioxide from sugar. In 1857, Th. Bail (I.) discovered the occurrence of budding mycelia (§ 219) in the family *Mucoraceæ*, and ten years later assumed the descent of beer yeast from *Mucor racemosus*. Doubt on this point seemed to him to be the more inadmissible, as he had noticed a weak alcoholic fermentation on submerging these budding *Mucor* mycelia in nutrient solutions containing sugar. Under the prevailing limitation of the term yeast, the name Mucor yeast was justifiable, though the same cannot be claimed for the generalised assumption, deduced from this observation, that a connection exists between the life history of yeasts and that of the higher fungi.

Soon afterwards a successful endeavour was made by M. Reess (I.) to become better acquainted with the yeasts, before disputing over their relationship to other fungi. Following up the previous discovery of the formation of ascospores (§ 247) in the cells of wine yeast and beer yeast, he recognised the importance of this phenomenon for the systematology of the yeasts, and did it justice by announcing this peculiarity as a principal characteristic of the now remodelled genus *Saccharomyces*, which he relegated to the *Eumycetes* group. Subsequently, the establishment of this characteristic led to a separation, which was objectionable from the standpoint of fermentation physiology: of the group of alcohol-producing budding fungi hitherto united under the common denomination "yeast," all those recognised as incapable of developing ascospores were rejected, and mostly, under the title "*non-Saccharomyces* of unknown systematic position," relegated to the group of *Fungi imperfecti*. The terms yeast and *Saccharomyces* ceased to be coincident. Many budding fungi capable of exciting alcoholic fermentation, *e.g.* several of the genus *Torula*, which still have some claim to be considered as yeasts, are therefore excluded from the *Saccharomyces* family because of their inability to put forth ascospores. On the other hand there are certain true *Saccharomycetes*, which are unable to excite alcoholic fermentation and therefore have no title to the name yeast (in the above sense of the term), *Saccharomyces membranæfaciens* being an example.

After the publication of Schwann's discovery, that of Reess was the first advance in the systematology of the yeast fungi; and it was left to E. Chr. Hansen to make the next move, viz. to separate the units (species, races, varieties) of the genus *Saccharomyces*, introduce experimental investigation into systematic description, and, for the first time, base this research on

really pure cultures. This he succeeded in accomplishing by degrees, so that the *Saccharomycetes* now form a large and well-defined family.

Of the objections laid against the determinations made by Reess, that put forward by Brefeld (V.) must be briefly considered. This worker observed, and reported in 1883, that the spores of smut fungi (*Ustilagineæ*, *q.v.*), when grown under special conditions, bud like yeast (Fig. 126), and that the process can be caused to repeat itself as often as desired. These yeast conidia (p. 22), as they were termed by Brefeld and others, are incapable of exciting alcoholic fermentation or producing ascospores. With regard to this deficiency, it was justly remarked by Reess (II.), that while Brefeld's observations increased the number of instances of yeast-like budding in the higher fungi, they by no means disproved the former worker's demonstration of the independence of the genus *Saccharomyces* and its allocation to the *Ascomycetes* group. Eight years later, Brefeld (IX.) repeated his hypothesis that the yeasts must be regarded as conidia from higher fungi (of a still unknown genus). He was reminded by E. Chr. Hansen (XVIII.) that the accuracy of this assertion still remained to be proved; and this latter worker also demonstrated, from existing data, that no connection between the life-history of the *Saccharomycetes* and that of any other fungi could possibly have been proved hitherto. Similar expressions of opinion have been uttered by A. de Bary, Zopf, H. Will, and others.

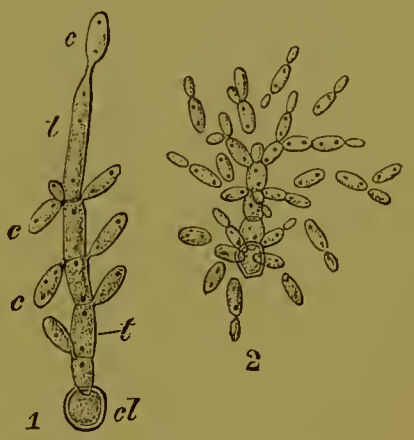


Fig. 126.—Ustilago carbo, the cause of smut in oats.

1. The spore *cl*, grown in a nutrient solution, has produced a polycellular mycelium (*t*), which has put forth yeast-like conidia (*c*). Magn. 450.
2. Chains of buds from these conidia. Magn. 200. (*After Brefeld.*)

As already stated on several occasions (§§ 220 and 243), the ascus can be distinguished from the sporangium by its more definite form and by the number, shape, and method of formation of its spores. This precision of form must be present in any fungus before the latter can be classified with the *Ascomycetes*. It is most decided in the more highly developed species, and becomes progressively less so as we descend the systematic scale; and, as a matter of fact, it is low (especially as regards the number of spores produced by the ascus) in the *Saccharomycetes*, which are the lowest of the *Ascomycetes*. In this genus, even in one and the same species, the number of spores is not always

the same in each ascus, and not infrequently an odd number is present. A similar lack of precision is also exhibited in other genera, such as *Ascoidea*, *Protomyces*, and *Thelebolus*. BREFELD (IX.) would associate all these into a special group, to which he gave the name *Hemiasci*, intending thereby to express the imperfect character of their asci, and that the development of the latter from the sporangia had been, as it were, arrested halfway. Therefore, according to his ideas, the *Hemiasci* formed a connecting link between the sporangiogenic *Phycomycetes* and the true *Ascomycetes*. Brefeld then endeavoured to effect an analogous separation between the other *Mycomycetes*, which, as we are aware, differ from the *Ascomycetes* by lacking the capacity for producing endogenous spores, and fructify by means of conidia. In the highest of these, namely the *Basidiomycetes*, the conidiophore is developed into a basidium (§ 384). On the other hand, in a number of *Mycomycetes* that do not exhibit ascofructification (viz. the *Ustilagineæ* and the *Tilletieæ*), the precision of the conidiophores is not so great as in the rest. Consequently they were separated — under the name *Hemibasidii* — from the latter, or *Basidiomycetes;* and, according to Brefeld's ideas, they formed an intermediate link between the *Phycomycetes* group (with conidial fructification) and the *Basidiomycetes*. They therefore constituted a branch of the *Hemiasci;* and these latter were grouped by Brefeld along with the *Hemibasidii*, to form an intermediate kingdom, under the new name of *Mesomycetes*. This conception, which is more fully detailed in a work by F. von Tavel, and according to which the *Saccharomycetes* should no longer be regarded as full-fledged *Ascomycetes*, was opposed by W. Zopf; but the final settlement of this highly complex question has not yet been reached. For us the *Saccharomycetes* will still continue to rank with the *Ascomycetes*, sub-class (order) *Gymnoasceæ*, and not as *Hemiasci*.

Any worker who attempts to trace a relationship between the *Saccharomycetes* and other fungi must agree with Reess's classification of the former with the *Ascomycetes*. Nevertheless, there is still room for diversity of opinion within the above limitation of the question. Thus, A. de Bary had already shown the great agreement, in structure and other particulars, between the *Saccharomycetes* and certain of the *Exoasceæ*, and remarked that the former might at once be ascribed to this latter group. Another matter worthy of attention is the question regarding any eventual connection between the *Saccharomycetes* and certain *Hyphomycetes* which have hitherto been included in the group of *Fungi imperfecti*. Some warrant may be accorded to such enterprise, though not without attention being drawn at the same time to the circumstance that a successful result must primarily be placed to the credit of these *Hyphomycetes*, since they would thereby obtain recognition as forming part of the

cycle of development of an *Ascomyces*, and consequently take a step upwards in the botanical system. This point, which may also lay some claim to the attention of earnest investigators, will be briefly touched upon in a later chapter dealing with *Dematium pullulans*. At present, on the other hand, we have to deal concisely with a couple of recent assumptions, or rather with the demonstration of their inapplicability.

In 1895 J. Juhler (I.) enlightened the world with the sensational report that he had succeeded in so influencing a species of *Aspergillus* as to cause it to develop *Saccharomyces* cells that produced alcohol. A communication shortly afterwards issued by Alfred Joergensen (II.), in whose laboratory the alleged discovery was made, explained that *Aspergillus oryzæ* was in question. They were quickly followed by E. Sorel (I.), who completed the beautiful circle by an alleged case of retrogression to the original mould fungus on the part of yeast cells stated to have originated from this *Aspergillus*. It was disturbing to observe the amount of perplexity induced by these publications, not among mycologists but on the part of a large number of fermentation technologists, as can be seen, for instance, in the case of a treatise by Eckenroth and Heimann (I.), who occupied themselves in a very similar manner with a *Penicillium*. In the interest of this branch it was necessary and indispensable that these hypotheses should be examined and refuted in all points by a number of workers schooled in botany. The credit of having shared in this task—a somewhat thankless one from the scientific standpoint—is chiefly due to Klœcker and Schiœnning (I. and II.), and also by O. Seiter (I.), C. Wehmer (VIII.), and J. Wortmann (IX.). All of them arrived at the harmonious conclusion that not under any circumstances, even the conditions of culture selected by Juhler, Joergensen, and Sorel, could any development of yeast cells take place from *Aspergillus oryzæ*, or conversion of *Saccharomyces* cells into this mould fungus. The same also applies to other species of *Aspergillus* examined with the same object by Klœcker and Schiœnning.

Consequently it may be averred that no proof, in any particular, has yet been afforded in support of the hypothesis that *Saccharomycetes* are derived from other fungi.

We therefore maintain that the distinguishing characteristic of the term *Saccharomyces* is a purely botanico-morphological one, namely, the capacity of the cell for producing ascospores. The method of formation of the latter will be fully described in § 247. All the *Saccharomycetes* are budding fungi, *i.e.* they vegetate in the form of a budding mycelium of the kind described in § 219. On the other hand, however, not every budding fungus is a *Saccharomyces*. The term yeast has two important characteristics: one morphological, the other physiological. In the

strictest sense the term is applied to such budding fungi as are capable of exciting alcoholic fermentation, and therefore the alcohol-producing Mucors are not to be classed as yeast; neither are the *Saccharomycetes* that lack fermentative power. Even in PASTEUR'S (III.) "Studies on Beer" no mutual limitation is placed on the terms *Saccharomyces* and yeast, but both are regarded as interchangeable, so that in many places we are unable to ascertain which is meant. The chief interest attaching to the yeasts is in respect of their practical utility. In many treatises on the physiology of the ferments the question whether they should be considered as *Saccharomycetes*, or as budding fungi of some other species, is left untouched. It is therefore impracticable to relegate the species there mentioned to one or the other group; and in such cases there remains no alternative but to speak generally of "yeast." This has also been done, and should be so understood, in the following paragraphs.

In laboratories where pure yeasts are cultivated for the purposes of the practical fermentation industry, it is very seldom that the rules of scientific nomenclature are adopted by supplementing the term *Saccharomyces* with a specific name, even when the species of the organism is known with certainty, the usual practice being to name the yeast in accordance with the locality of origin. Thus the Johannisberg wine yeast No. 1, illustrated in Fig. 124, was obtained from the sediment of a young Johannisberg wine. "Saaz yeast" is a bottom-fermentation beer yeast isolated by P. Lindner from the stock yeast of the Saaz brewery (Bohemia). This yeast will be frequently mentioned later, on account of its very low power of attenuation. The very high-attenuation "Frohberg yeast" originated at Frohberg's brewery, Grimma (Saxony). In many instances the yeast is simply given a number, under which it is registered in the laboratory collection (living herbarium) and is cultivated further. Thus, the distillery yeast known to distillery technologists and mycologists under the abbreviated title "Race II.," is the second of a series of yeasts given out by the Berlin Experimental Station, for practical testing in respect of their utility, the species in question finally proving superior to the others.

It may be remarked casually that a proper discrimination between the three expressions: budding fungus, *Saccharomyces*, and yeast, is often lacking in medical treatises dealing with a pathogenic budding fungus. Some of these are known to produce illness, even attended with fatal results, when they find the conditions of development favourable in the body in which they have made their habitat. The literature on this matter has been collected by J. RAUM (I.), and in a monograph by O. BUSSE (I.), as also in the different volumes of P. Baum-

garten's *Jahresbericht*. These species are oftentimes spoken of as *Saccharomyces*, or yeast, although their connection with this genus, or their faculty of exciting fermentation, is more or less doubtful. This remark applies to the so-called *Sacch. farciminosus Tokishige*, *Sacch. neoformans*, and *Sacch. lithogenes Sanfelice*; *Sacch. sphæricus* and *Sacch. ovalis van Hoorn*, &c. One species recognised as exciting fermentation, and consequently to be classed as a yeast, though not as yet proved to be a true *Saccharomyces*, is a budding fungus discovered by O. Busse, and observed by him to produce, in the human body, a kind of general debility (*Saccharomycosis*) which may terminate fatally. That certain true yeasts are pathogenic when artificially introduced into the arterial circulation, was proved in 1892 by Hueppe, in the course of experiments with Rauenthal wine yeast and young porpoises. This result has been repeatedly confirmed since, notably by L. Rabinowitsch, in a series of experiments with about fifty stocks of different species and origin, seven of which proved pathogenic.

§ 245.—Bottom Yeasts.

If a number of flasks be charged with a clear nutrient solution, of a kind favourable to the growth of yeast and containing a fermentable sugar, and each of them be inoculated with a trace of a pure culture of different yeasts, such as are used in brewing, distillery work, vinification, &c., the cultures being then kept at room temperature for a couple of days, it will be found that cell reproduction and fermentation—manifested by the appearance of turbidity and gas bubbles—will occur in all. It will thereafter soon be possible to separate the flasks into two groups, according to the appearance presented. In the one group the yeast crop developed from the sowing will remain almost entirely within the liquid throughout the entire period of fermentation, and mostly at the bottom even from the start. Yeasts of this kind are termed **bottom yeasts**, and excite **bottom-fermentation**, the yeast crop being sedimental.

In the other group the fermentation is very brisk and attended with the formation of large quantities of froth (head); and in the earlier stages a larger or smaller number of the new cells are raised to the surface by the ascending bubbles of gas, and remain there—provided the vessel be high enough to prevent frothing over—until fermentation is terminated and the froth breaks up, whereupon they sink down to the bottom of the liquid and increase the sedimental deposit. This kind of fermentation is termed **top-fermentation**, and the yeasts producing it are called **top-fermentation yeasts**. Typical examples of bottom-fermentation yeasts are afforded by the Munich lager-beer yeasts. On the other hand, the most highly

developed top-fermentation yeasts are the species forming the bulk of the pressed yeast prepared by the old (Viennese) process. This pressed yeast consists exclusively of cells raised from the mash by the head, and of the daughter-cells of same, since it is impracticable to separate from the mash such cells as are left therein. These two extreme types of yeast are connected by a number of intermediate grades. The question of another, and fundamental, difference between top- and bottom-fermentation yeasts will be discussed in the paragraphs relating to melibiase.

As soon as the primary fermentation is manifestly at an end, let us take a trace of the sedimental yeast from each of the flasks, thin each sample down with a drop of water on a glass

FIG. 127.—Saccharomyces cerevisiæ I. Hansen.
Cells from the sediment of a young culture in beer wort. Magn. 1000. (*After Hansen.*)

slide, cover it with a cover glass, and examine it under a high power (250–500) in the microscope. In many of the specimens the cells will be found globular or oval in shape; speaking generally, most of the beer yeasts and brandy yeasts will present this appearance. Now because, since the time of Meyen, the name *Saccharomyces cerevisiæ* has been usually applied to beer yeast, it has gradually become the custom to say of yeasts exhibiting approximately globular or oval cells of large dimensions, that they are of the **cerevisiæ type.** Fig. 127 gives an example of this class, namely a top-fermentation yeast isolated by HANSEN (XII.) from the stock yeast (of which it formed the main constituent) of a top-fermentation brewery in Edinburgh and called by him *Sacch. cerevisiæ I.*

The sedimental yeast in other flasks will be found to differ from the foregoing, inasmuch as it contains cells which, instead of being globular or oval, are elliptical in shape. Yeasts of this kind were invariably found in fermenting must by REESS (I.), who called them *Sacch. ellipsoideus*, which specific name gradually became enlarged to a morphological designation, so that we

therefore speak of this or that yeast as being of the **ellipsoideus type**, meaning thereby solely, in the first place, that the cells of such species are generally elliptical in shape and rather smaller than those of the cerevisiæ type. Fig. 128 gives an example in the *Sacch. ellipsoideus I.* obtained by HANSEN (XII.) from the surface of ripe grapes. This type is exhibited by many species of wine yeast; and it is therefore easy to understand why, in the absence of a method of pure culture and the consequent impossibility of determining the existence of divers species,

FIG. 128.—Sacch. ellipsoideus I. Hansen.
Cells from the sedimental yeast of a young culture in beer wort. Magn. 1000.
(*After Hansen.*)

Reess's appellation of *Sacch. ellipsoideus* very soon became a synonym for wine yeasts in general. This custom is, however, now no longer justifiable, since we are nowadays acquainted with races of wine yeasts whose cells cannot be classed with the ellipsoid type, but are globular or elongated. Conversely, not every yeast of the ellipsoideus type is a wine yeast. An example of such a divergent yeast—though in respect of the shape of the cells in the yeast sediment it is almost identical therewith—was isolated by HANSEN (XII.) in 1883, from the stock yeast of the Tuborg brewery in Copenhagen, namely the so-called *Sacch. ellipsoideus II.* This yeast occurred there, in association with *Sacch. Past. III.*, as a technically pathogenic yeast, inasmuch as it produced disturbances in the brewing, or rather tended to form haze in the trade casks or bottles.

The third group, consisting of the remainder of the flasks used in our experiment, differs from the other two, inasmuch as the cells of the young sedimental yeast are of elongated form, something the shape of a sausage or a short tube with closed

ends, and in some species slightly constricted in one or two places. Cells of this kind were noticed by PASTEUR (XII.) during his researches on wine. They were also found by REESS (I.) in the secondary fermentation of certain wines examined by him; and this worker named them *Sacch. Pastorianus* in honour of the French scientist. Cells of this type have been more frequently observed by subsequent investigators, and this specific name has been gradually modified into a morphological term. When it is said of a yeast that it exhibits pastorianus forms, or is of the **Pastorianus type**, the term merely implies that under normal conditions the sedimental yeast of the species in question chiefly forms cells that are sausage-shaped, and not globular, oval, or elliptical. An example of this kind is afforded by *Sacch. Pastorianus I.*, illustrated in Fig. 129. This species was discovered by HANSEN (II. and XII.), in 1880 and 1881, in the air at the Alt-Carlsberg brewery, Copenhagen, and was introduced into the literature, under the above name, by him in 1882, after he had succeeded in proving that it had also crept into the stock yeast of this brewery, imparting to the resulting beer an obnoxious bitter by-flavour and a smoky smell. It is therefore a virulent **pathogenic yeast** (in the technical sense).

It would be a great mistake on the part of the reader to assume, from the foregoing sketch of the three main typical forms of yeast cells, that each species of yeast invariably assumes the same form. This is not the case, a powerful influence on the form being exerted by the conditions of cultivation. This last fact was unknown, and indeed undiscoverable by the methods in use, at the time Reess set up his specific classification based solely on the form of the cells. Thus, up to the year 1882, it was thought that the bottom-fermentation yeast used in brewing always consisted of the one single species *Sacch. cerevisiæ;* and it was not until 1883 that E. CHR. HANSEN (XII.) showed that we have to reckon with a large number of species, and that consequently the names *Sacch. cerevisiæ*, *Sacch. Pastorianus*, *Sacch. ellipsoideus*, &c., could henceforth be merely used as group names. Since that time, no small portion of this worker's investigations has been devoted to the question of the dependence of cell form on the conditions of cultivation, and to the elucidation of the fact that, morphologically, the character of a species of yeast does not reside in the form of the cell alone, but also in the manner of its dependence on the external conditions of which it is the result. If these conditions be known to a certain extent, then the form of the cells constitutes a very valuable and fairly reliable indication. Since, like other manifestations of vitality, the form of the cells is a resultant of two components, namely, inherited properties and the sum of all the external influences, it is evident that, even if the uniformity of the latter conditions could be made absolutely certain, the

former reason alone would preclude the expectancy of perfect regularity in the cells of a culture. Moreover, owing to the imperfect state of our knowledge, both in chemistry and physics, the production of absolutely identical conditions of existence in two cultures started at different times, is unattainable. Again, even when working with a single cell, it will be found that the daughter-cells, grown in one and the same nutrient medium, differ among themselves; in one of them one of the inherited properties latent in the mother-cell makes its appearance, whilst in another of the cells other inherited properties preponderate.

FIG. 129.—Sacch. Pastorianus I. Hansen.
Cells from the sedimental yeast of a young culture in beer wort. Magn. 1000. (*After Hansen.*)

It is necessary to lay emphasis on this point, because it only too often happens that a beginner in the cultivation of yeast feels compelled to lose all faith in his knowledge when he observes that a pure culture, prepared from a single cell strictly according to all the rules of the method, consists of cells all more or less different in size and form. Now there is nothing special about such variability, which is exhibited by bacteria and all other living organisms, and that too in no less a degree than with the yeasts. Nevertheless, within the limits afforded by the aforesaid difficulty of control, one and the same species grown under the same conditions will give cells of approximately uniform shape, say, for instance, that of the sedimental yeast of the Cerevisiæ type.

That under these conditions, it is actually possible to trace differential characteristics, may be explained by the aid of the two drawings shown below, both of them representing samples from the sedimental yeast of a culture in beer wort, at the close of the primary fermentation. Fig. 130 is the so-called Carls-

berg Bottom-Yeast No. 1, the first yeast prepared by the pure-culture method, and by means of which E. Chr. Hansen introduced his pure-culture method into practice, at the Alt-Carlsberg brewery, in 1883. It was isolated as the principal constituent of the same stock yeast, which was found to be infected with the aforesaid *Sacch. Pastorianus I.* A characteristic feature of this yeast is the preponderance of pointed oval cells, those of purely globular form being very much in the minority. Elongated cells also are very rare. On the other hand, the Carlsberg Bottom-Yeast No. 2, shown in Fig. 131, is characterised by the more rounded appearance of its cells, and by the occurrence of unusually large, or giant, cells, one of which can be seen on the left of the illustration. The appearance of these

FIG. 130.—Carlsberg Bottom-Yeast No. 1.

Cells from the sedimental yeast at the close of primary fermentation. Magn. 1000. (*After Hansen.*)

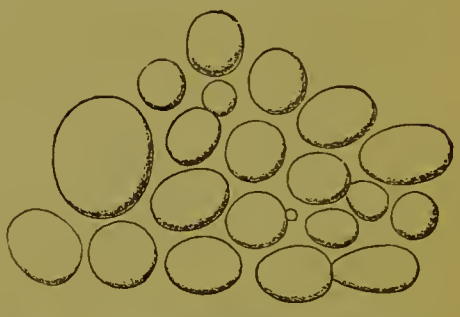


FIG. 131.—Carlsberg Bottom-Yeast No. 2.

Cells from the sedimental yeast at the close of primary fermentation. Magn. 1000. (*After Hansen.*)

giant cells is specially remarkable in some species, and then forms a good indication. Thus, BEYERINCK (XX.) found large cells attaining as much as 20 μ in diameter, in old agar-agar cultures of a budding fungus known as *Sacch. Kefyr*, which he had isolated from the Kephir to be described in our final chapter, whereas the other cells measured only 5 to 6 μ.

The **sedimental yeast** found deposited at the bottom of the fermenting vessels at the close of primary fermentation, in bottom-fermentation breweries, is drawn off—after the removal of the young beer—through an orifice in the bottom of the tun, and is collected in a vessel wherein it is washed with water, to be afterwards stored under ice water until required for pitching a subsequent brew. This yeast, it may be stated, is a highly diversified mixture. Apart from the possible presence of several species of yeast and bacteria (*sarcina*, &c.), it also contains sundry other ingredients, the most important of which are: First, salts of lime, chiefly the oxalate, immediately recognisable under the microscope by its octahedral, rhombohedral or flat tabular crystals. These have been precipitated during fermentation. Their origin is only to a small extent

attributable to the metabolism of the yeast; they also occur in unfermented wort, and in wort-gelatin, and, it may be remarked in passing, disturb the beginner when he examines such a gelatin plate under a low power (30 to 100) before inoculating with micro-organisms, or employing it for starting a single-cell culture. Secondly, the yeast mixture contains precipitated hop resins, in the shape of very small globules, sometimes united as aggregations; they give the resin reactions, and therefore assume a handsome red coloration in presence of alcoholic tincture of alkanna root. Thirdly, there are the so-called glutin bodies, which are fine globules of an albuminous nature, originating in the malt and precipitated from the wort at the low temperature of the fermenting cellar. They have formed the subject of some deep researches by H. Will (III.). Fourthly, certain dark brown fragments, which are mostly looked on as hop resins by practical brewers, but in reality are said by H. Will (IV.) to give all the reactions for albumin. When present in large quantity they form a source of trouble in brewing, by enveloping the yeast cells and rendering these latter inoperative. The upper layer of the sedimental yeast in the vat containing the beer in condition for racking, is specially rich in such extraneous admixtures, and is consequently rather dark in colour. This portion is generally removed in advance and thrown away, before the underlying "white" yeast is drawn off. Fifthly come mucilaginous matters of different kinds (§§ 254 and 255), which have been excreted by or extracted from the yeast cells. Sixthly are residual fragments of the mashed materials, hops, lupulin granules, and not infrequently aphides, and the like.

It has already been stated that all the samples examined were taken from fresh cultures, *i.e.* cultures in which the primary fermentation was just terminated and the deposited yeast crop was of recent date. On the other hand a different appearance is presented by the cells of a sediment that has lain for some time under the fermented liquid—that is to say, in old laboratory cultures, or the sludge found at the bottom of lager-beer storage vats, and therefore consisting of cells that have been exposed to the influence of the supernatant beer for some considerable time (often several months). Under these circumstances a large number of elongated cells of the *Pastorianus* type are produced, even in yeasts that are ordinarily of decidedly cerevisiæ type. This is a very useful thing for the beginner to know, in order that he may not be disheartened on finding a number of cells of the suspicious *Pastorianus* type among the sludge of a vat, the beer in which was fermented with a pure culture yeast. Provided the brewing operations have been conducted in a cleanly manner, the process of spore analysis, described in § 247, will quickly reassure him of the absence of infection.

Up to the present the composition of the **deposit in wine** and the "wine yeast," in the sense used by tartaric acid manufacturers, has not been so thoroughly examined as is the case with the sedimental yeast of breweries. Next to yeast, the chief constituent is cream of tartar, which is gradually precipitated in the crystalline form by the increasing amount of alcohol in the fermenting wine.

When exposed to very unfavourable conditions, the yeast cell assumes a true involution form. Cells of this kind, resembling amœbæ in contour, are found, for example, in very old gypsum-plate cultures, or similar cultures on wort gelatin. They have been drawn by P. LINDNER (VI. and X.). Many cells that have become exposed to unfavourable conditions in sedimental yeast are able to withstand the same and survive uninjured, because they have previously laid up a considerable store of material and greatly strengthened their membrane. Under these circumstances they become permanent cells, which may be regarded as gemmæ (§ 223). These forms will be further discussed in §§ 246, 249, and 251.

§ 246.—Film Formation.

Yeast cultures, started in the manner foreshadowed at the commencement of the preceding paragraph, are able to exhibit another form of development. If the samples have been taken with every care, all infection being prevented, and the vessels again closed with their germ-proof plugs of cotton-wool—which, however, do not exclude air—all that is then necessary is to store them in a quiet corner of the laboratory for some time (several weeks or even months), to find that **film** has developed on the surface of the liquid. The structure and physiological behaviour of this film will now be described.

This form of development originates in cells, which, thanks either to the presence of some fatty excretion adhering to the outside of the membrane, or to some albuminous or resinous deposit acquired from the nutrient medium, are able to float on the surface of the liquid when the latter becomes quiescent after fermentation has terminated. A good opportunity for these cells to remain afloat is afforded at the places where the surface of the liquid is in contact with the walls of the (round) culture vessel. Hence, it is in such positions that the development of the film will be earliest observed, in the form of a whitish ring. In many instances this ring is by no means complete at first. but appears in the form of a series of patches, *i.e.* **colonies of cells**, which gradually increase and then meet, coalescing to a closed ring. In other cases the annular formation is less fully developed, and the growth of the film proceeds from small

colonies or islets of yeast which have originated on the surface of the liquid itself. Other conditions being equal, the dimensions attained by the film vary according to the species of yeast.

The form of the cells from which the film is constructed differs in general from that of the sedimental cells, by attaining greater **longitudinal development** (up to 150 μ and over), whilst the transverse measurement is often less than in the cells of sedimental yeast. The second characteristic is a more or less **abundant branching**. An example is shown in Fig. 132.

The time required for the development of the film to become manifest differs, other conditions being equal, with the species of the yeast, and is longer the lower the temperature of the culture. According to a series of determinations made on species of *Saccharomyces* by HANSEN (XVI.), the time required for *Saccharomyces cerevisiæ I.* at 33° to 34° C. is about 9 to 18 days; at 20° to 28° C., about 7 to 11 days; at 13° to 15° C., about 15 to 30 days;

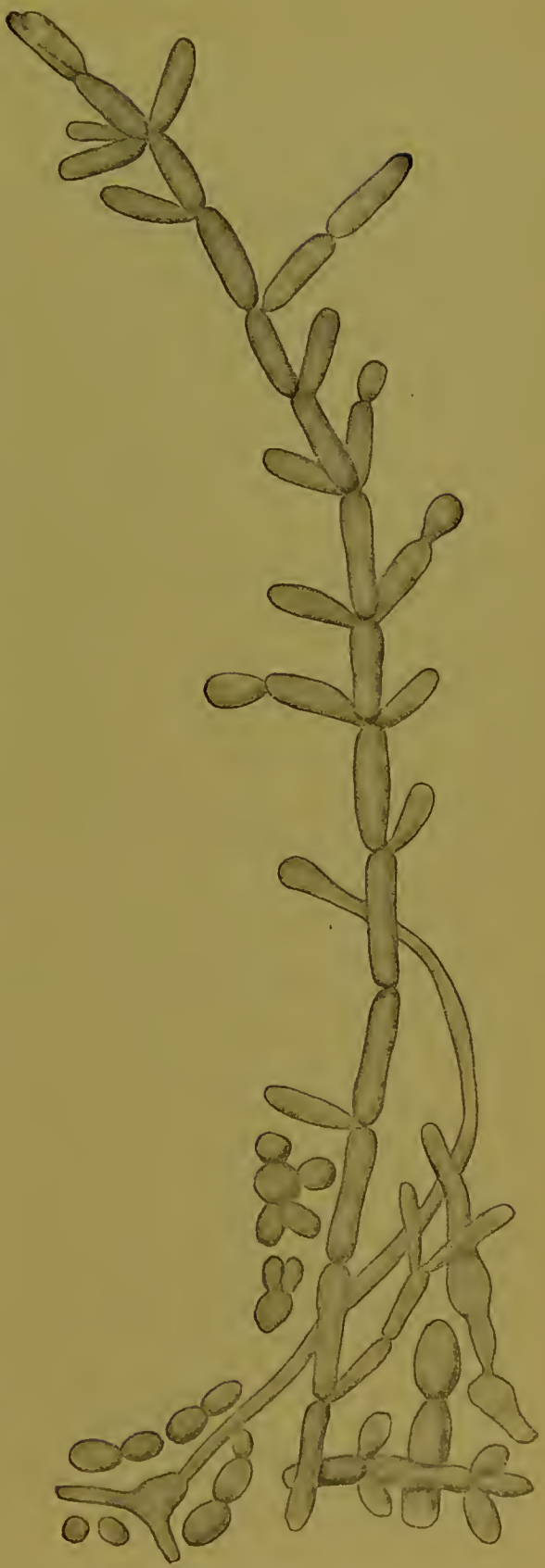


FIG. 132.—Saccharomyces ellipsoideus I. Hansen. Cells and chains of cells from the film of an old culture in beer wort. Magn. 1000. (*After Hansen.*)

at 6° to 7° C., about 2 to 3 months. No formation of film occurred above 34° C. or below 5° C.

These limits of temperature, which also apply to all the other species examined, are therefore more restricted than those wherein the yeast is able to bud and incite fermentation. Film formation is therefore precluded in the fermenting cellars of bottom-fermentation breweries, the temperature here being, wherever possible, maintained between 0° and 2° C. It has already been shown by Hansen, and confirmed by H. Will (VIII.), R. Aderhold (I.), and other workers, that the time elapsing before the film makes its appearance, and the dimen-

Fig. 133.—Sacch. Past. II. Hansen.

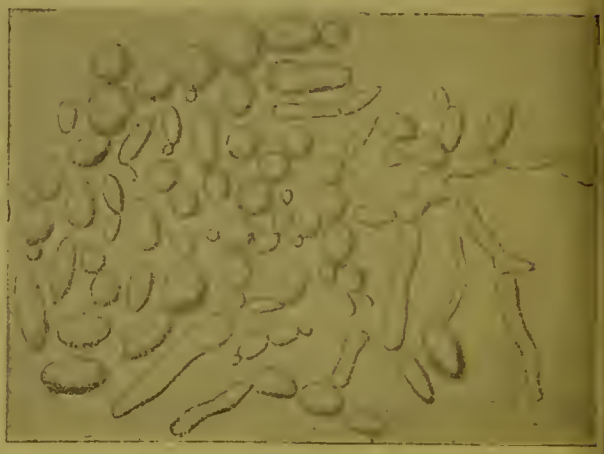


Fig. 134.—Sacch. Past. III. Hansen.

Cells of film grown on beer wort at 20° to 28° C. Magn. 1000. (*After Hansen.*)

sions attained by the film, are very greatly dependent on the conditions of cultivation (composition of the nutrient solution, and also in a high degree on the method of sterilisation, supply of air, &c.).

In some cases morphological peculiarities in the film cells afford a means for differentiating the species. With *Sacch. Past. II.* and *Sacch. Past. III.* this can be recognised in a beautiful manner, and at the same time a fresh instance is afforded of the dependence of cell form on temperature. The first-named, weak, harmless, top-fermentation species was isolated by Hansen from the air of the Carlsberg brewery. The other, which is of decidedly top-fermentation character, was obtained as a pure culture from a Copenhagen lager-beer suffering from haze, and was recognised as the cause of the malady. It is difficult to distinguish between them by the form of the cells present in the sedimental yeast, both being very similar to *Sacch. Past. I.* (see Fig. 129). The same also applies to the component members of their films grown at 20° to 28° C. and illustrated in Figs. 133 and 134. The case is, however,

different—as HANSEN (XVI.) has shown—when the two have been allowed to form films at 13° to 15° C., since under these con-

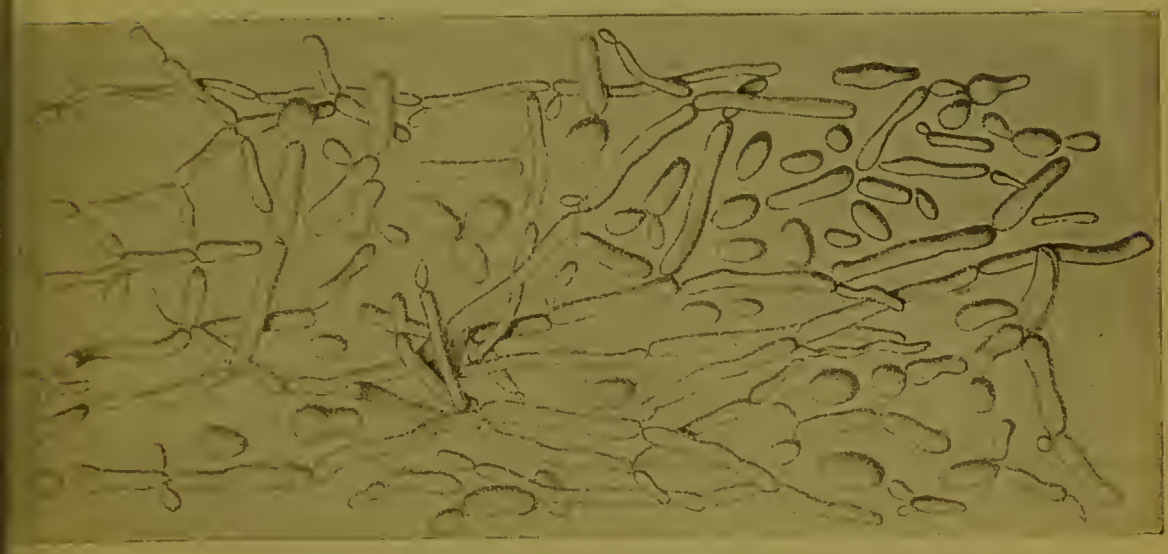


FIG. 135.—Sacch. Past. III. Hansen.
Cells in film grown on beer wort at 18° to 15° C. Magn. 1000. (*After Hansen.*)

ditions one of them, *Sacch. Past. III.*, exhibits a number of highly elongated cells (Fig. 135) with lateral buds, whilst those of *Sacch. Past. II.*, on the other hand, retain very much the same form as when grown at 20° to 28° C., *i.e.* only slightly elongated, and even globular. An essential precaution for this comparison is the use of young films, since when the films are very old the No. II. species exhibits elongated cells just like those of No. III. Similar conditions in this respect obtain between *Sacch. ellipsoideus I.* and *Sacch. ellipsoideus II. Hansen.*

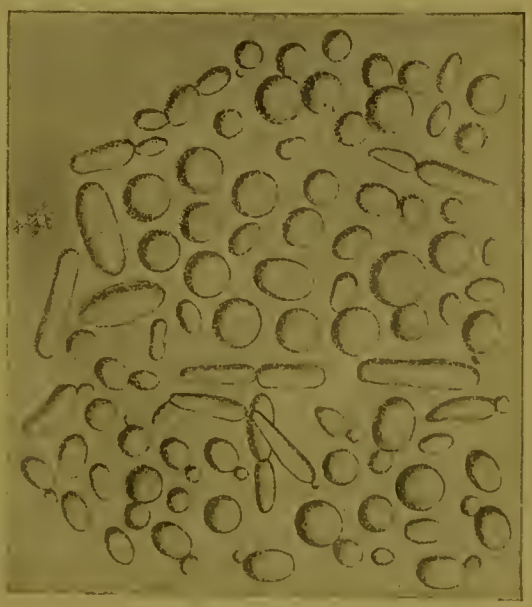


FIG. 136.—Saccharomyces Past. II. Hansen.
Cells in film grown on beer wort at 13° to 15° C. Magn. 1000. (*After Hansen.*)

The faculty of developing a film on the surface of suitable nutrient solutions is shared by nearly all the budding fungi,

both *Saccharomycetes* and *non-Saccharomycetes*. Of these latter we shall deal (in the last section), under the generic name *Mycoderma*, with a special group, the species of which are widely distributed and grow spontaneously on the surface of wine or beer when the latter are exposed to the air, a quick-growing, wrinkled skin (mould film) being formed. Air being highly essential to *Mycoderma*, these organisms normally grow exclusively in the form of a superficial film on the nutrient solution. Owing to this peculiarity they are the cause of disturbance in researches into film-formation in the true yeasts, when the latter are not grown as pure cultures but contaminated with the very abundant *Mycoderma*. On this account objection may be raised against the reports of Reess (the first to observe the production of films by true *Saccharomycetes*), and also those of Pasteur (III.). In 1876 the latter characterised as ærobic or mould yeasts the film developing on the surface of fully fermented wort. He halted between two opinions with respect to this phenomenon, one being that it was a special (*i.e.* ærobic) condition of development of the beer yeast residing at the bottom of the fermented liquid; whilst the other looked on the film as composed of extraneous cells undesirably present with the sowing. It was not until Hansen applied pure cultures to these investigations that a decision could be formed on this point.

The question of the convertibility of bottom-fermentation yeast into top-fermentation yeast, and *vice versa*, is also touched by the foregoing explanations. Pasteur was of opinion that the "ærobic" cells constituting the film that had developed it at the close of primary fermentation in his cultures inoculated with (impure) bottom-fermentation yeast, were capable of exciting top-fermentation when transferred to a fresh nutrient solution. He even gave a recipe by means of which the brewer could prevent any such undesirable conversion of the stock yeast. This theoretically and practically important question was afterwards taken up by Hansen, who found that the descendants of the films of all the species of bottom yeasts examined by him in this connection, invariably produced nothing but bottom-yeast cells when inoculated into fresh nutrient solutions, even when exposed to a temperature (26° C.) very favourable to the progress of top-fermentation.

The true *Saccharomycetes* can be separated—though not very sharply—into two groups: one of which does not form films until the primary fermentation is terminated and the sedimental yeast has all come down; whilst in the other group growth proceeds on the surface from the commencement, and indeed in many cases exclusively so. An instance of this latter kind is afforded by the *Saccharomyces membranæfaciens*, first discovered by E. Chr. Hansen (VIII.) in the mucinous

discharge (§ 248) from elm, and later in well-water by J. KOEHLER (I.).

The separate stages of the development of film were closely investigated in four species of bottom-fermentation yeast by H. WILL (VIII.). At the outset no difference can be detected between the cells of the sedimental yeast and those retained floating on the surface by flakes of albumin and residual fragments of the "head," and from which the development of the

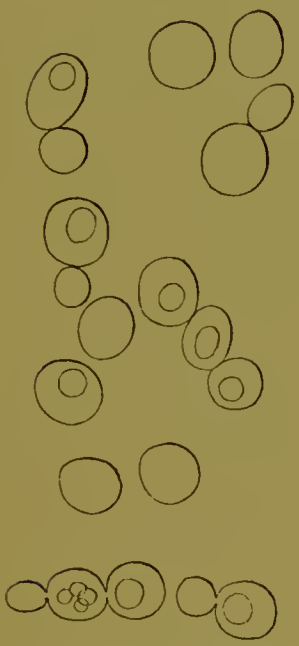


FIG. 137.—Cells of sedimental yeast from a wort culture of—

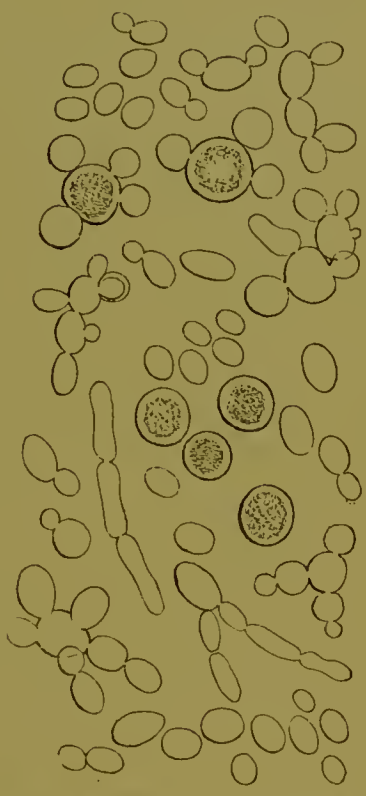


FIG. 138.—Film cells of the first generation, and permanent cells from the film of a wort culture of—

Bottom-fermentation beer yeast No. 93 of the Munich Brewing Station. Magn. 750. (*After Will.*)

yeast islets originates. Later on, however, it is observed that these floating cells produce daughter-cells, the chief feature of which—as may be seen from a comparison of Figs. 137 and 138—is that, instead of appearing singly or in pairs on the mother-cell, as they do in the sedimental yeast, a number are formed simultaneously thereon. They are also much smaller (*e.g.* only 7 μ as compared with 10 μ) than those in the sedimental yeast, are oval or sausage-shaped, and in turn produce similar daughter-cells, the whole remaining connected

together. These cells form a new and special generation, which Will terms the **first generation of true film cells**, since with their appearance the formation of the film begins. In addition to these there quickly form other cells, which are characterised by considerably thicker membrane and an abundant content of glycogen and fat. These prove to be true permanent cells, both on account of their anatomical structure and their physiological behaviour, since they alone retain vitality and are capable of development when the cultures are old and all the other cells and the film have perished. The yeast ring, which grows about the same time, is specially rich in these permanent cells. They soon produce highly elongated, sausage-shaped or tubular cells, which in turn behave in a similar manner and also produce analogous lateral daughter-cells, the result being the formation of many-membered chains of the kind shown in Fig. 132. Will terms the members of these bands, film cells of the second generation. The older the film the more luxuriant do they grow, and the farther do the film cells of the first generation retire to the background. At a later period, partition walls are formed—more or less abundantly in the different species—in these elongated cells; and similar septa are also found in the chains of buds resulting from the germination of the permanent cells in wort. A view of these is given in Fig. 139.

These chains of elongated cells partake of the character of an articulated mycelium. The capacity for producing such was first positively demonstrated by Hansen, and proves that the *Saccharomycetes* belong to the *Mycomycetes*, or *Eumycetes* with septate mycelia. Their position within this sub-kingdom was then, as already stated, indicated by their capacity for producing ascospores, which will be discussed in the next paragraph. In coloured nutrient media, such as beer wort and wine, the progress of film development is accompanied by a bleaching action, *i.e.* the disappearance of colouring matters. In this manner the colour of a wort can be slowly changed from dark brown to straw yellow.

The film cells also differ strongly in their chemico-physiological behaviour from those of the sedimental yeast. The latter still develop in presence of an extremely low oxygen tension, and devote their chief energy to the decomposition of sugars. On the other hand, the metabolism of the film cells is indissolubly connected with the presence of a copious supply of oxygen. According to the results of investigations conducted on this point by B. Raymann and K. Kruis (I.), they oxidise, to carbon dioxide and water, the alcohol continuously formed in the fermenting underlying solution, and degrade the albuminoids therein to amides and ammoniun salts of organic acids. Formic acid and valerianic acid are also formed. Hence

in this case the fermentative action of the sedimental yeast is replaced by respirative activity.

When submerged in fresh nutrient solution, these film cells produce vegetations, which finally behave just like normal sedimental yeast. The rate of morphological change and adaptation of physiological character differ with the species of yeast. In some, as was ascertained by H. Will (VIII.), the characteristics appertaining to the film cells remain unimpaired in the first new generations, and in specially conspicuous cases several recultivations (repeated tranferences of the crop to fresh nutrient solution) are required in order to produce a sedimental yeast equal in all respects to the original ancestors of the film cells used. Notice should also be taken, *e.g.* of an observation on this point by Ed. Kayser (VI.). Further consideration will be given in subsequent paragraphs to this behaviour, from the standpoint of the theory of variation.

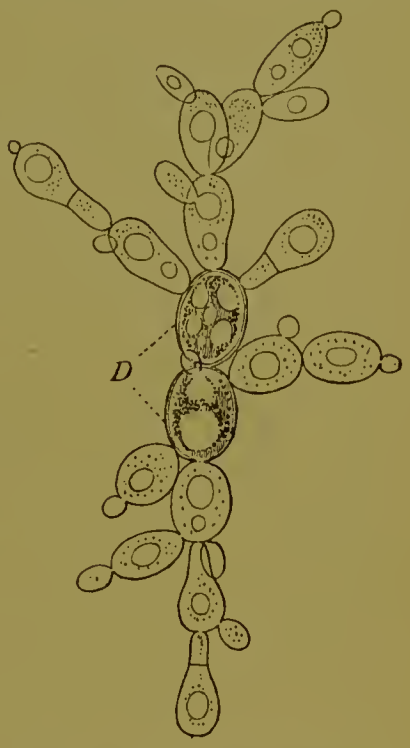


Fig. 139.—Pair of Permanent Cells from the yeast ring of a six-months-old wort culture of Munich bottom-yeast No. 2, and germinated to a well-developed chain of buds in a drop of wort on a microscope slide in sixty-four hours at 10° C. A septum has formed inside three of the members of the chain. Nearly all the cells exhibit one or two vacuoles, and the two permanent cells (D) show an even larger number. Magn. 750. (*After Will.*)

At present we have to deal with the consequences connected with the practical cultivation of yeast, namely the restriction of film vegetation and the exclusion of cells derived therefrom. To effect this object it is necessary to keep the stock yeast in the laboratory under such conditions as are unfavourable to the development of film, without being at the same time inimical to the sedimental yeast. The appearance of the former may be counteracted by frequent transferences of the cultures to fresh nutrient solutions, and by keeping the culture at low temperatures. According to Hansen, the best storage medium for prolonged use is a 10 per cent. solution of saccharose. In this event, however, the sowing should not amount to more than a trace. When, from any cause, the only yeast culture available for fulfilling an order is one that is already covered with a film, the same is suitable for direct transference to the large repro-

duction vessel, but must be first freshened up by preparing a re-inoculation, which in turn is used to inoculate a fresh nutrient solution as soon as development is in full swing. The operation is several times repeated, according to circumstances, until one is able to assume that the film cells present in the first inoculation of sedimental yeast have been entirely suppressed. The beginner cannot be too strongly advised not to regard the task of yeast cultivation as completed by the preparation of the pure cultures, but rather to keep the latter under constant supervision, examination and care. Neglect of these precautions, and, in the case under consideration, the use of sedimental yeast containing film cells, may, under certain circumstances, lead to irregularities in the progress of fermentation on the large scale, diminution in the quality (flavour, &c.) of the product, and hence to unpleasant consequences for the yeast cultivator. Cases in point have been recorded by A. Joergensen (VII.).

This, however, must not be held to imply that the film cells are the cause of all the unwelcome alterations that may appear in beer yeasts. On the contrary, other forces are here in operation; and from this side also, as already mentioned, we arrive at the wide field of variation in the yeast cell, a domain in which, as will be shown in a later chapter, Hansen, by his extensive experimental researches, has been our pioneer. Moreover, it should be recalled that Raymann and Kruis (I.) were able, by means of yeast derived from old film cells, to produce good beer that could not be distinguished from that obtained by the aid of normal yeast. This harmonises with the results obtained by Alb. Klœcker (privately communicated to the author) with Carlsberg bottom-yeast No. I. and No. II., *Sacch. cerevisiæ I. Hansen*, Marienthal yeast, and Will's No. 2 stock yeast.

Will's observations bring to mind the flying yeast (Flughefe) so dreaded by the brewer, *i.e.* yeast cells which are of smaller size than those of the sedimental yeast, and, instead of settling, continue to swim in the beer, and thus retard clarification. This presumptive relation has not yet been more closely investigated, but the researches of Hansen and others have placed beyond doubt that this phenomenon is in many cases attributable to the presence of wild yeasts. Another point that requires closer examination is the part played, in the maturing of beer, by the film cells produced within the liquid. Finally, investigation from this point of view is also desirable on the problem of the cause of flocculence in the process of making pressed yeast by the new, so-called aeration or wort process, which differs chiefly from the Viennese method (§ 255) in the thick mash being replaced by a clear mash as nutrient medium; this, after pitching, being well roused by aeration, whereby the reproduc-

tion of the cells is strongly stimulated, accelerated, and increased. When fermentation is at an end the contents of the fermenting vessel are cooled and drawn off into large, flat clarifying pans, where the yeast crop settles down, and, after the removal of the supernatant liquid for distillation, is washed with water and finally forced into filter presses, where it is brought into saleable condition. Occasionally the deposition of the yeast crop in the clarifying pans, and therefore its separation from the liquid, is obstructed by a so-called flocculence, which is characterised by the continued re-ascension of flocculent aggregations of cells from the deposit. The phenomenon has been described by STENGLEIN and JOERRES in "Alcohol" (1892, p. 218), and also by O. DURST (I.).

§ 247.—The Ascospores.

The first observation of the ascospores in yeast cells was made by TH. SCHWANN (II.). He pointed out that these fungi reproduce in two ways: one being by the process known as budding, and the other by the formation, within the parent-cell, of daughter-cells, which are liberated when the membrane of the parent-cell opens. After this phenomenon had been described more closely by J. DE SEYNES (I.) in 1868, it was also observed a year later by M. REESS (III.) in cultures of beer yeast on boiled sections of carrots, &c. He found the process of development coincide with that of certain low *Ascomycetes*, and therefore classed these forms as ascospores, calling the mother-cells asci, and for this reason relegating the *Saccharomycetes* (in 1870) to the position of the lowest family among the *Ascomycetes*.

The earliest accurate investigations into the conditions under which this sporulation occurs were carried on by E. CHR. HANSEN (XII.), and, apart from the general biological results, led to the important fact that we have here a reliable means, hitherto lacking, of separating the genus *Saccharomyces* into its species.

The conditions influencing the production of ascospores in the *Saccharomycetes* are given below: (1) To obtain energetic sporulation, it is necessary that the sample should consist of young and well-nourished cells. (2) The supply of air must be abundant. (3) The medium must be moist. (4) The temperature of the environment must be maintained within certain limits. (5) Within these limits the time required for the occurrence of sporulation is a function of the temperature. (6) Between the two extreme limits at which sporulation is still possible is an optimum temperature corresponding to a time minimum. The maximum temperature for sporulation is some-

what lower, and the minimum temperature rather higher, than for the phenomenon of budding.

To examine the individual conditions more closely. That the time within which sporulation occurs should be a function of the temperature, requires no further analysis; but careful attention should be bestowed on the point (in 1) as to the condition of the cells, this being the prime factor determining the time limit. The time required for ascospores to be developed by any given species of *Saccharomyces*, kept at any given temperature, differs according to the physiological condition of the cells themselves. Hence, if it be desired to produce sporulation (unconditionally) in any given species, all that is necessary is to take cells that are in vigorous condition—a state attainable by repeated preliminary transferences into fresh nutrient solution. The case is, however, different when it is a question of determining the time required for sporulation to make its appearance at one or another temperature. In such event, it must be borne in mind that this time limit is a function, not merely of the temperature, but also of the physiological condition of the species under examination; consequently this latter factor must be eliminated in order to enable the influence of the former to be determined. Experience has shown that sporulation occurs earliest and most certainly when the cells have reached the culminating point of their reproductive (budding) and fermentative activity; and it is therefore in this condition that they should be employed for the experiment in question. On this account the cells to be examined for the time limit of sporulation should be subjected to the following preliminary treatment: the sample is sowed in sterilised beer wort and left to stand for several days at room temperature, Pasteur flasks being the best vessels for the purpose. A portion of the resulting sedimental yeast is transferred to fresh sterile beer wort and kept therein for twenty-four hours at 25° C., the fresh deposit being afterwards freed as carefully as possible from the supernatant liquid, and employed for starting the spore cultures.

One example will suffice to show the necessity of taking the condition of the cells into consideration. It is afforded by Hansen's experience, and relates to *Sacch. Pastorianus I.* The culture was first conducted for a few days at room temperature, after which the sedimental yeast was retransferred, in the above manner, to two flasks, one of which was kept for twenty-four hours, the other for forty-eight hours, at 26° to 27° C. before starting the spore cultures. The following figures show the time required for the commencement of sporulation in the two cases:—

Sacch. Pastorianus I. Hansen.

Sporulation occurred at	After previous Cultivation at 26° to 27° C. for:—	
	24 Hours.	48 Hours.
29° C. . . .	At the end of 27 hours.	No Sporulation
28° to 27.5° C.	,, ,, 24 ,,	At the end of 36 hours.
23.5° to 23° C.	,, ,, 26 ,,	,, ,, 30 ,,
15° C. . . .	,, ,, 50 ,,	,, ,, 54 ,,

The sample previously treated for forty-eight hours gave no sporulation at a temperature (29° C.) at which the other produced spores in abundance. Apparently this unfavourable effect is attributable to the increased alcohol content produced by the longer period of fermentation. The relation in question has also been observed by other workers, *e.g.* by H. Mueller-Thurgau (III.), A. Aderhold (I.), &c. The (artificially effected) temporary or permanent loss of sporogenic capacity of yeasts will be dealt with in the chapter on variation.

The best means of obtaining the moist medium and copious access of air essential to sporulation, is afforded by the **gypsum block** proposed by Engel (I.) and suitably improved by Hansen. This block is a truncated cone, about 3 to 4 c.m. in height, which is prepared, by means of an ungreased mould of sheet-iron, from a mixture of 8 parts, by volume, of powdered, calcined gypsum and 3 parts of water. After the block has become perfectly dry through long exposure to the air, it is placed in a covered glass basin enveloped in a double layer of filter-paper, and sterilised in the drying oven for an hour at 115° C. When cold, the yeast under examination is sown over the upper surface of the block, by means of a platinum loop, previously sterilised by heating to redness. The glass basin is then charged with sufficient sterilised water to quickly moisten the block completely, from below upwards, and in addition leave a layer of water about 1 c.m. deep in the bottom. The cover glass should not fit quite tight, but is preferably supported on an uneven bearing, so as not to exclude the admission of air. The arrangement of the whole is shown in Fig. 140, about one-half or one-third the real size. The block, in its basin of water, is then placed in a thermostat, maintained at the desired temperature for the experiment.

The nature of the material composing the moist medium also influences the time limit of sporulation. Since the necessary cleaning of the gypsum block, by brushing with water after each experiment, gradually destroys the block itself, H. Wichmann (III.) proposed to replace gypsum by solid cubes of fire-

brick. It was, however, shown by J. Chr. Nielsen (I.) and Alb. Klœcker, that sporulation took longer on this substance than on gypsum; on the other hand, equal results with the latter are furnished by the clay cubes first employed by H. Elion (III.). Th. Bowhill (I.) uses gypsum blocks of the same shape (a cylinder, cut through slantwise) as the slices of potato employed for streak cultures in test glasses; and he likewise uses these glasses. No advantage accrues from the substitution of filter-paper for gypsum blocks recommended by E. Wasserzug (II.).

The conditions with regard to access of moisture and air are also fulfilled by streak cultures on solid nutrient substrata; and, as a matter of fact, ascospores are formed under such circumstances—as was first observed by Hansen, and afterwards by other workers. For example, the part of the gypsum block can also be played by a damp wall (in the brewery) towards the yeast sprinkled thereon, or by a damp filter-bag, &c., for yeast gaining access thereto from the air. Again, as is shown in Fig. 141, spores may occasionally be observed in the development of film; but these are merely exceptional instances. Their occurrence was vainly sought by H. Will in bottom-fermentation beer yeasts. On the other hand these organs are, naturally, not lacking in the films of the comparatively few *Saccharomycetes* that grow almost exclusively in the form of film and not as sedimental yeast, a special instance being afforded by *Sacch. membranæfaciens*. Sporulation has also often been observed in the interior of liquid cultures: by Hansen in repeatedly aerated cultures of several species in yeast water, and in *Sacch. Ludwigii* grown in 10 per cent. saccharose solution; also by H. Will (VIII.) in a wort culture of his bottom (beer) yeast No. 93; by P. Roeser (II.) in a 1 *per mil.* solution of peptone; and by Hauteefeuille and Perrey (I.) in a culture of wine yeast in must at 28° C. When the nature of the question at issue necessitates absolute purity of the spore culture, Hansen makes the sowing on a thin layer of water at the bottom of a flask, and not on a gypsum block in a glass basin.

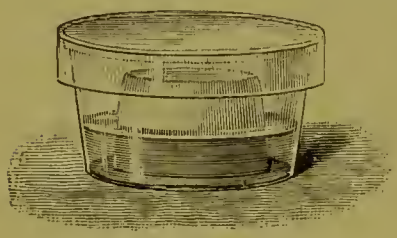


Fig. 140.—Gypsum-block Culture.

With regard to the inception of sporulation we know comparatively less than we do of the conditions under which the phenomenon occurs. In general it may be said, according to the determinations made by E. Chr. Hansen (XXVIII.), that the cells do not resort to sporulation until they are unable to reproduce further by budding. Both old and young cells are capable of producing spores, even such as have not put forth any

buds at all. In the case of Wortmann's wine yeast Johannisberg II., for example, this result can be obtained by placing the young cells in a saturated aqueous solution of calcium sulphate, whereby budding is suppressed and sporulation begins. Compare what has been stated in the last paragraph but one of § 223.

The progress of development in the ascospores will now be followed in the case of *Saccharomyces cerevisiæ I. Hansen.* If, at the end of about twenty-four hours after the streak has been laid on the block and the latter placed in the thermostat at 25° C., a small portion of the yeast streak be scratched off with a clean glass rod or a needle, distributed in a single drop of water, and examined

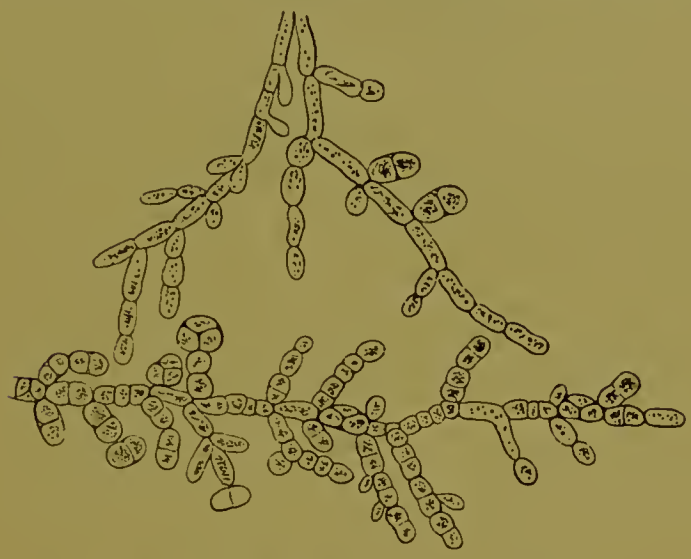


FIG. 141.—Wine Yeast from Walporzheim.

Assemblage of buds from an old film; some of the members have produced spores. Magn. 800. (*After Aderhold.*)

with a strong power (300 to 500) under a cover glass, decided indications of incipient sporulation will be observable in a larger or smaller number of the cells. The plasma will be found to have separated itself into a number of balls, as shown at *a–d* of Fig. 142. Each of these globules soon becomes covered with a membrane, so that ripe spores (*f–j*) will be visible after another twenty-four hours. With regard to the behaviour of the cell nucleus during this period, fuller particulars will be given in § 250. It may be remarked that a portion of the plasma in the parent-cell (ascus) remains over, and holds the spores somewhat together.

A careful sorting of the samples will quickly show that considerable differences exist with respect to the dimensions of the spores, and that too not merely between those in different cells

but also in those present in one and the same ascus. The number (2 to 9) of the spores in the different asci will also be found to vary. What has already been said of the cells of sedimental yeast, also applies to the ascospores: neither form nor dimensions is a reliable indication of species, and therefore these characteristics cannot serve unaided for their specific differentiation. It is only in special instances that this is possible, namely when a species of the group *Sacch. anomalus* is present. The first representative of this group was discovered by HANSEN (XVII.) in a Bavarian stock yeast, and it was he who gave it the foregoing specific appellation. This new species differs from those we have known hitherto, inasmuch as the ascospores, instead of being perfectly globular, are minus a more or less considerable portion of the sphere; and as the margin of this flat or slightly arched terminal surface is surrounded by a projecting border, the whole ascospore has the form of a hat (see Fig. 143). In the course of time other species producing similar ascospores, but differing in other respects, have been discovered, so that at present we have to deal with a whole group of such Saccharomycetes, which will be reported on more fully in a later section. More will also be said later on about the special method of sporulation observed by H. SCHIŒNNING (I.) in *Schizosaccharomyces octosporus* and possibly to be regarded as a sexual act.

FIG. 142.—Sacch. cerevisiæ I. Hansen.
Ascosporulation. Magn. 1000.
(*After Hansen.*)

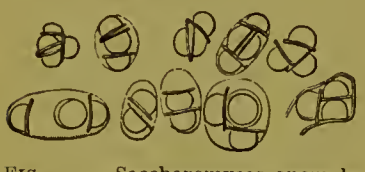


FIG. 143.—Saccharomyces anomalus Hansen.
Ascospores. In three instances these have already been set free. In one the membrane of the parent cell is just breaking; in the remainder this has not yet happened. Magn. 1000. (*After Hansen.*)

When it becomes necessary to express in figures the time limit for sporulation, the moment selected is that at which the first appearance of spores becomes visible. To wait until the period of ripening, is, on the other hand, impracticable, there being no reliable means of determining when that state is attained. Taking the first-named as the critical moment, HANSEN (XII.) was the first to determine the relation between temperature and the time limit of sporulation of six species of *Saccharomycetes*. Afterwards a series of workers made similar determinations with a large number of species: H. WILL (VIII., IX., XVII.), J. CHR. HOLM and S. V. POULSEN (II.), with European beer yeasts; A. LASCHÉ (IV.), with American beer yeast; L. MARX (I.), ED. KAYSER (VII.), and the latter along with G. BARBA (I.),

on French wine yeast; R. Aderhold (I.), on German wine yeasts; A. Nastukoff (I)., on Russian wine yeasts; and W Seifert (I.), on Austrian wine yeasts; also by E. Kayser (III.), on French fruit-wine yeasts; and by Chr. Grœnlund (I.), J. Chr. Nielsen (I.), and Alb. Klœcker (I.), on several species still to be mentioned. The main object of such investigations is the determination of the minimum, maximum, and optimum temperature of sporulation; and several complete series of similar data have been compiled. The relation between the height of the thermometer and the time limit of incipient sporulation in a given species may also be graphically expressed by plotting the temperatures as abscissæ and the corresponding time-limit values as ordinates of a rectangular system of co-ordinates, and connecting the points of intersection by a line. Such lines are mostly termed sporulation curves, in the literature of the subject.

The percentage ratio of the number of sporogenic cells to the total cells present differs greatly according to the species, origin, kind of previous nutrition, &c., but in most species fails to attain even approximately to 100. In many of the cultivated yeasts, *e.g.* five out of thirty-two species of bottom-fermentation beer yeasts examined by Lasché (IV.), no success has, so far, attended the attempts made to bring about sporulation; consequently for the present these cannot be regarded as *Saccharomycetes*.

On the basis of his experimental results in connection with sporulation, already alluded to, Hansen has worked out a method of biological analysis for brewery stock yeasts; and this method has been further developed, especially by J. Chr. Holm and S. V. Poulsen (I. and II.).

The term "culture yeast" is applied to such yeasts as have been cultivated for practical use in fermentation technology, and have then been employed therein. More than this is not implied by the expression, nor does it apply in any way to their origin. On the other hand, the yeasts met with wild in nature are termed generally wild yeasts. Many of these are harmless, though others, as already mentioned in § 245, are capable of producing disturbances on gaining access to the operations of brewing, &c., and are then spoken of as (technically) pathogenic. Examples of this class are afforded by *Sacch. Past. I. H.*, which imparts a repulsive bitter taste to beer; also *Sacch. Past. III. H.* and *Sacch. ellips. II. H.*, which produce haze. As a further deduction from the observations of Hansen that the wild yeasts in general form ascospores more quickly and abundantly than the bottom-fermentation (culture) beer yeasts, Holm and Poulsen have shown that the latter can be classified into three groups according to their behaviour in this respect. The members of the first group remain sporeless, both after seventy-two hours at

15° C. and after forty hours at 25° C. Those of the second group, whilst remaining sporeless after forty hours at 25° C., exhibit spores after seventy-two hours at 15° C. Finally, the members of the third group behave in an exactly converse manner to those last mentioned. On the other hand, the wild yeasts exhibit spores under both sets of conditions (15° C. and 25° C.) within the time limits specified (seventy-two and forty hours respectively), or even much earlier. Consequently the desired differentiation can be obtained in all cases by making parallel tests at 15° C. and 25° C.

According to Hansen's observations—which we shall have to deal with in the paragraphs treating of mixed sowings—the wild yeasts that have crept into the pitching yeast or the wort first make their appearance in large quantities towards the end of primary fermentation in the upper portions of the contents of the fermenting vessel. Consequently at this period a sample should be drawn from this part in a glass; the suspended yeast cells must be left to subside and then immediately transferred to the prepared gypsum blocks, which are maintained at the temperatures of 15° C. and 25° C. respectively. At the end of forty and seventy-two hours respectively a sample is taken of each, and should spores be detected in either or both series, then the presence of wild yeasts is demonstrated. When the brewery is unprovided with a suitable laboratory and an expert, a sample of the sedimental yeast must be sent to a laboratory, a drop of the yeast being dried on blotting paper in the manner described in a later paragraph dealing with this matter. Owing to the abundant sporulation of the wild yeasts, this method is very decisive, Holm and Poulsen having by this means succeeded in detecting the presence of as little as about $\frac{1}{2}$ per cent. of added wild yeast (one or other of the above-named three pathogenic yeasts) in twenty different species of bottom-fermentation beer yeasts. For practical use this is sufficiently delicate. On the other hand, to be of any value for testing yeast in a pure-culture apparatus, a method must be absolutely reliable and capable of detecting and isolating even the slightest trace of infection. With this object the yeast from the apparatus is subjected to a preliminary treatment, by means of which the amount of any wild yeast present in the sample is increased. This matter will be further described in a subsequent paragraph treating of the influence of organic acids on yeasts.

Differences also exist between the cultivated bottom-fermentation beer yeasts and the wild yeasts, in connection with the structure of the ascospores. Those of the latter are relatively smaller, and their contents are homogeneous and of high lustre, whereas the ascospores of the culture yeasts mostly exhibit vacuoles and granulation, and the membrane is clearly discernible.

Note to Binder.—This table to be inserted near the end of § 247.

CONJOINT INFLUENCE OF TEMPERATURE AND TIME ON THE SPORULATION OF CERTAIN SACCHAROMYCETES.

THE FIRST DEFINITE APPEARANCE OF SPORULATION OCCURS AT x° C. AFTER DAYS (d) OR HOURS (h):

No.	Species x:	½°C.	1°	2½-3°	3°	4°	4½°	5°	5½°	6°	7°	7½°	8°	8½°	9°	9½°	10°	10½°	11°	11½°	12°	12½°	13°	13½°	14°	15°	15½°	16°	16½°	17°	17½°	18°	19°	19½°	20°	21°	21½°	22°	22½°	23°	23½°	24°	24½°	25°	25½°	26°	26½°	27°	27½°	28°	28½°	29°	29½°	30°	30½°	31°	31½°	32°	32½°	33°	33½°	34°	35°	36°	37°	37½°	38°	39°	41°C.	Spores. Dimensions.	Spores. No. in each Ascus.	Observer.	No.
1	Sacch. cerevisiæ I. Hansen														nil					10d									65h		50h									27h				23h										20h							23h		25h	29h		nil				2·5-6 μ	1-5	Hansen (XII)	1
2	Sacch. Past. I. H.	nil			14d						7d			5d			89h									50h						35h									26h								24h			27h		30h			nil													1·5-5·0 μ mostly 1·5-3·5 μ	1-10 mostly 1-4		2
3	Sacch. Past. II. H.	nil			17d						7d									77h						48h				36h										27h				25h					34h			nil																		2-5 μ	1-7		3
4	Sacch. Past. III. H.					nil								9d				7d										53h		44h								29h						28h			30h		35h			nil																		2-4 μ	5-10		4
5	Sacch. ellips. I. H.					nil						11d						4½d								45h						33h												21h									23h			36h			nil											2-4 μ			5
6	Sacch. ellips. II. H.					nil							9d						5½d													42h												27h								22h					23h			27h	31h		nil							2-5 μ			6
7	Sacch. membranæfaciens H.			nil							6-7d		4½-5d																															17¼-18h						17¼-18h				17-18h	17-18h				18h	19-21h			nil									Nielsen (I)	7
8	Sacch. Ludwigii H.			nil						13-14d			7-8d																															20-21h						19-21h				18-19h	18-20h			19-21h				nil								3-4 μ	1-8		8
9	Sacch. anomalus H.			nil						13-14d			7d																															18-20h						17½-19h				17-19h	18-19h			19-21h				nil								2-3 μ	2-4		9
10	Sacch. Ilicis Grönlund												nil			20d						10d					3d																	22h					20h									18h				20h		22h			nil					Grönlund (I) or Holm and Nielsen.	10
11	Sacch. Aquifolii Grönlund												nil				15d				8d							3½d				48h				44h			31h							29h		28h		29h						nil																	11
12	Will's Yeast No. 811					nil							9d			6d					4½d						41h			38½h								20h		17h				14½h												11½h						11h	12h		15h			23h	nil	1-5 μ mostly 3·5 μ	1-4	Will (IX)	12
13	Will's haze-forming Yeast	nil			21d		14d						8d				6d				5d		102h			74h						53h	46h			36h						29h		31h				35h							48h			nil												2-4 μ	1-4		13
14	Bottom Beer Yeast No. 2 (Of the Munich Experimental Station.)										nil								9½d				123h		113h	83h				69h		63h	49h			39h		36h		34h				31h						32h				44h		47h						nil										Will (VIII)	14
15	Do. No. 6										nil								11d					5d		82h		73h		62h		58h			50h	46h		42h		39h				36h		35h				33½h				45h		51h						nil											15
16	Do. No. 7																		nil				5½d		83-107h	62½h						48½h			41½h			35½h						32h		31½h				32h				50h		nil																		16
17	Do. No. 93										nil						10½d		5½d				4½d		83½h	74h				54h			42h		41h					36½h				34h						31h				48h		nil																	17
18	Sacch. Zopfii Artari																																		70h					42h						45h						21h						nil													1-4 mostly 2	Artari (I)	18
19	Sacch. Jörgensenii Lasché											nil									4d																							17h		20h						nil																		1·0-2·5 μ	2-4 mostly 2-3	Lasché (I)	19

The examination of the top-fermentation beer yeasts, distillery yeasts, and pressed yeasts by this process is a more delicate operation, because they sporulate much earlier and more abundantly than the bottom-fermentation yeasts; for instance, a species from the Munich Brewing Station, examined by Will, produced spores in fourteen hours. Nevertheless, according to JØRGENSEN (VIII.), the test is applicable if low temperatures (12° C.) be employed. The wine yeasts sporulate still more rapidly, 46 out of 58 examined by Marx being found to produce spores within twenty-four hours at 25° C. Similar results were obtained by Aderhold and by Nastukoff. The proposed supercession of spore analysis by the small-drop culture method introduced by P. LINDNER (XV.), will be referred to later, in a paragraph dealing with pure culture applied to yeasts.

When the gypsum block cultures are left exposed for a long time they become infested with all kinds of amœbæ, which gain access from the atmosphere and grow by devouring the yeast, as was observed by P. LINDNER (VI. and X.). In such event, sporocysts, the permanent form of these animalculæ, can be discovered on the blocks.

§ 248.—Germination of Saccharomycetes Spores. Their Resistance to Heat. Pasteurisation.

REESS (I.) was the first to observe the germination of *Saccharomycetes* ascospores, and more extensive and precise investigations were afterwards conducted by E. CHR. HANSEN (XVII.), whose reports constitute the main basis of the information now given.

FIG. 144.—Saccharomyces cerevisiæ I. Hansen. Germination of spores. For explanation see text. Magn. 1000. (*After Hansen.*)

Speaking generally, the germination of spores proceeds in one of four different ways. An example of the first and most usual method is afforded by *Sacch. cerevisiæ I. Hansen.* The ascospores of this yeast are capable of germinating as soon as ripe, unless prevented by unfavourable external circumstances. The presence of **water** is an **indispensable condition**, large quantities of this liquid, and of any nutrient substances present, being taken up by the germinating spores, which thereupon swell up to a considerable size. If they are still enveloped by the parent cell, this latter is stretched and assumes a somewhat angular contour, as shown at Fig. 144 at *a* and *d*. The residual plasma in the parent cell is compressed flat between the spores, but reacts on the latter, and forces them into a polyhedral shape so

long as the membrane of the parent-cell continues to hold firm. Consequently the ascus looks as though divided into compartments. In some instances (see *g*) a coalescence of the spore membranes occurs at the points of contact. In the case illustrated, the parent-cell contained three spores fused into one by the coalescence of their membranes. On pressing the cover glass this fused aggregation was expelled from the parent-cell, the membranes of the three compound spores bursting simultaneously, each in one place. A similar case is illustrated at *e*, but with this difference that here the parent-cell contained four spores, one of which remained behind after the application of pressure, the parent-cell having contracted to its original dimensions. Even without artificial aid the membrane of the parent-cell finally gives way under the growing pressure and liberates the spores, which then put forth buds (*f*) and henceforward behave

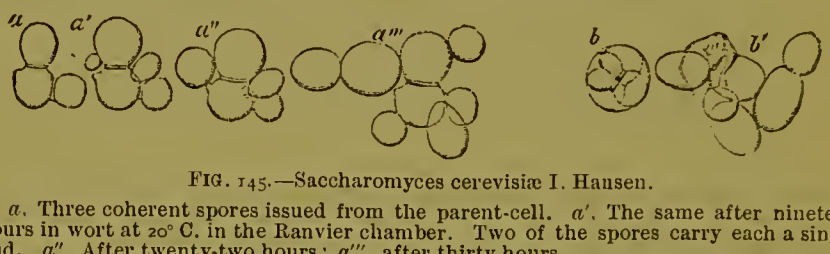


FIG. 145.—Saccharomyces cerevisiæ I. Hansen.

a. Three coherent spores issued from the parent-cell. *a'*. The same after nineteen hours in wort at 20° C. in the Ranvier chamber. Two of the spores carry each a single bud. *a''*. After twenty-two hours; *a'''*. after thirty hours.

b. A parent-cell with four spores; the two below are partly covered by the upper pair. *b'*. After eighteen hours (as with *a'*). The membrane of the parent-cell has become torn, and hangs, like a veil, on the colony of cells formed from the spores. Magn. 1000. (*After Hansen.*)

like vegetable cells. The extent of subsequent budding naturally depends on the external conditions (nutrient media, admission of air, temperature, &c.). Two examples are shown in Fig. 145. In some instances the septum formed by the coalescing membranes of two spores is absorbed and a fusion of the contents of both spores occurs. The ascospore germination of all the yeasts employed in brewing, distilling and wine making follows this first typical course.

An example of the second method of spore germination is afforded by *Sacch. Ludwigii*. Various genera of deciduous trees, the oak in particular, frequently exhibit a mucinous flux which is readily perceptible with a little care. In the earliest stage of the complaint there exudes from the bark—both from frost cracks, knot-holes, and apparently uninjured places—a white froth, the smell of which is enough to show that alcoholic ferments have contributed to its formation. For more detailed investigations on the phenomenon we are indebted to FR. LUDWIG (II.). This exudation, which may also prove of interest to foodstuff chemists in connection with birch wine, was recognised by him as the field of activity of various species of micro-organisms, the occurrence and action of which he traced in

several directions. One of the fungi present and identified as exciting alcoholic fermentation in this exudation, was then examined by E. Chr. Hansen (XXIX.) and named *Sacch. Ludwigii* in honour of its discoverer. This fungus is interesting in many respects, *inter alia* as a very fine example of mycelial film and septation in the cells of same. At present, however, we have to do with another peculiarity, namely, the behaviour of its endospores during germination. These spores, instead of putting forth rounded buds of a shape similar to their own, as in the type just described, produce an elongated, sausage-shaped promycelium of the kind illustrated in Fig. 146, g''', h'''. This promycelium it is that puts forth the buds, and it therefore

Fig. 146.—Saccharomyces Ludwigii Hansen.

d. Two spores, germinating on the one side (d'), fusing (d''), growing into a promycelium (d'''), putting forth a bud (d'''') at the crown, and separating by the development of a septum (d'''''), whereupon one of them (d'''''') assumes a rounded form, which procedure is repeated several times (d''''''').

g, *h*. Two spores, each growing to a tube which produces daughter-cells independently, without coalescence. Magn. 1000. (*After Hansen.*)

constitutes an intermediate stage between the bud cells and the spore. Contrary to the usual procedure, the yeast cells of normal shape produced by this mycelium are not liberated as the result of constriction, but by the development of a septum at the place where separation afterwards occurs. This is shown at g''''. Very often, however, the promycelium is not the result of the germination of one single spore, but of the coalescence of two germ tubes to form one new cell. This process, one of actual cell fusion, is shown d–d''''''', where the two spores can be seen germinating on the one side, each in an elongated form (d'), after which the two fuse together (d'') to a single cell, which in turn grows out at one side to form a promycelium (d''''). A daughter-cell (d''''') then separates and quickly assumes the lemon shape (d'''''') characteristic of this fungus. The cell fusion occurring in this instance differs from the previously described type in two ways: there it was the exception,

here it is the rule ; there it took place between the spores themselves, here only in the germ tubes proceeding from the spores. This fusion is not of invariable occurrence, but is occasionally hindered by the tubes (*g'''*) taking different directions from the start, or by the premature withering of one or the other of them ; or sometimes only one is formed, the adjacent cells being prevented from germinating by circumstances, *e.g.* age.

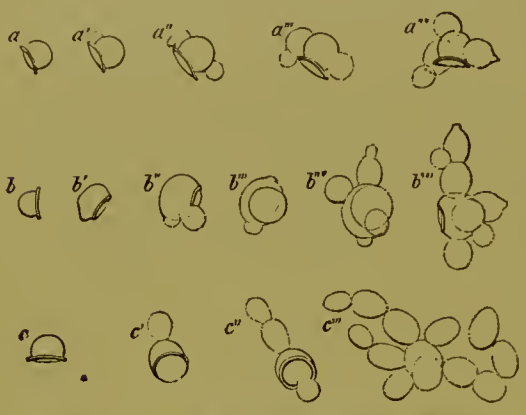


FIG. 147.—Sacch. anomalus Hansen. Spore germination.

a. A spore at the commencement of germination, with the brim turned towards the left ; *a'* is the same after seven hours, one bud having been formed ; *a''* is the same after twelve hours, a second bud appearing on the spore ; *a'''* is after fifteen hours ; and *a''''* after twenty hours.

b. A spore with the brim turned towards the right ; *b'* the same after ten hours ; *b''* after twenty-one hours ; *b'''* at the end of twenty-four hours ; *b''''* after twenty-five hours ; *b'''''* after twenty-seven hours, the spore with its daughter-cells having turned through an angle of 180° in the nutrient solution.

c. Is a spore with the brim turned downwards ; *c'* the same after eight hours ; *c''* after ten hours ; *c'''* after twenty-four hours.

Magn. 1000. (*After Hansen.*)

Apart from *Sacch. Ludwigii*, this method of spore germination is only known to occur in one other species of *Saccharomyces*, which was discovered by J. BEHRENS (VIII.) on fresh hop cones.

The third method of germination is found in the group *Sacch. anomalus*, and differs from the first type solely in the influence exercised by the peculiar shape of the spores. Three examples are illustrated in Fig. 147. These relate to sowings of spores taken from old gypsum-block cultures and grown in beer wort in the Ranvier chamber, the top row being kept at 28° C. and the other two at 23° C. The germinating spore swells up, and gradually puts forth a number of buds at various points of its surface, these in turn reproducing by budding. During this operation the hat-brim-like projection round the spores disappeared in the middle series (*b*), but remained in the other two (*a* and *c*).

A fourth type of spore germination is exhibited by *Sacch. guttulatus*. This fungus is an invariable inhabitant of the contents of the stomach and intestines. It also occurs in the excrement of the full-grown rabbit, and was first discovered therein by R. Remack in 1845. It was classed along with the yeasts by Robin in 1847, under the name *Cryptococcus guttulatus*, and finally recognised as a *Saccharomyces* by Buscalioni, in 1896, as the result of a searching investigation. In this species, which does not bud until about 37° C., but is capable of sporulation at about 14° C., and thus forms in this respect an

exception to the rule given in the preceding paragraph, the sporulation proceeds in the manner described in §§ 57 and 58. As was first demonstrated by ARM. WILHELMI (I.), the contents of the elongated germinating ascospore envelop themselves in a new membrane, the resulting germ then splitting the old membrane, either at the end or laterally. In the act of swelling it also bursts the membrane (if still intact) of the parent-cell, escapes, and immediately begins to bud. An illustration of this process is given in Fig. 148.

The spore, however, does not invariably germinate, but, as was ascertained by HANSEN (XXVIII.), may, under certain circumstances, itself play the part of an ascus and develop ascospores (of the second order) in its interior, instead of reproducing by germination and budding. This end may be attained by allowing the ripe spore to swell up in a solution of sugar, and then transferring it to a saturated aqueous solution of gypsum.

FIG. 148.—Saccharomyces guttulatus.

Two ascospores, the contents of which have enveloped themselves with a new membrane whilst still enclosed in the intact membrane of the parent cell (ascus). The old spore membrane was ruptured terminally in the one spore and laterally in the other. Both germs still partly invested by the old spore membrane. Magn. 1066. (*After Wilhelmi.*)

The ascospores of *Saccharomycetes* exhibit greater powers of resistance to external influences than is possessed by the vegetative cells. Reference will be made in subsequent paragraphs to cases wherein the extraction of moisture, drying, or chemical stimulants, come under consideration as influences of this nature. At present we are mainly concerned with the effects of temperature, and that too in presence of liquids. HANSEN (XII.) was the first to point out that the spores of *Sacch. ellipsoideus II.* perish in five minutes when kept in sterilised water at 66° C., though they survive this period at 62° C. On the other hand, the vegetative cells taken from a two-days old culture in beer wort at 27° C. perished within five minutes on immersion in water at 56° C., although they could stand 54° C. In the case of *Sacch. Past. I.*, the corresponding values obtained were 62° and 58° C. for the spores, and 54° and 52° C. for the young vegetative cells. The resisting power of old vegetative cells, the permanent cells especially, approximates more closely to that of the ascospores. Greater tenacity of life was exhibited by a species (*Sacch. Zopfii*) isolated by Zopf from the sugar juice in a Silesian beet-sugar works, which species was more closely examined by A. ARTARI (I.), and does not perish below about 67° C., under identical conditions. And still greater resistance is offered by the *Saccharomyces* mentioned in the preceding paragraph as WILL'S (IX.) No. 811 yeast, which was discovered in a beer of repulsive, bitter, irritant flavour, of which malady it was recognised as the cause. When kept in beer wort at

70° C., an exposure of half-an-hour was required to kill the vegetative cells, whilst the spores withstood the influence of 75° C. for the same period. Antithetical to this is *Sacch. Jœrgensenii*, which was discovered by A. Laschié (I.) in a very turbid American, so-called temperance ale, and quickly perishes at temperatures above 30° C. Similar experiments were made with French wine yeasts by E. Wasserzug (II.) and southern wine yeasts by Ed. Kayser (VII.), the latter of whom found the fatal temperature for spores to be about 5° C. higher than for vegetative cells.

In order to ensure stability in fermented beverages it is sufficient to so far weaken the yeast cells by warmth as to preclude subsequent reproduction, and thus practically eliminate them. To attain this end, experience has shown that the necessary influence must be less stringent than is required for killing the cells; and the case is facilitated when the liquid contains substances which, like alcohol and acids, exercise an injurious effect on yeasts and bacteria at a somewhat higher temperature than the normal. These latter conditions are fulfilled in the case of wine, beer, and wine-must, and an explanation is thus afforded of the fact that these liquids can be converted into a stable condition by a gentle heat, considerably below boiling point. The earliest application of such a process is traceable to the Japanese. According to O. Korschelt (I.), the Japanese rice beer, or *saké* (already mentioned in an earlier paragraph), is made to keep through the hot summer months by warming, a practice that has been pursued for more than a century. The first to adopt such a method in Europe was Scheele in 1782 (§ 11); and Appert, in 1810, recommended the warming of wine, in corked bottles, to 75° C. It was, however, soon found that this treatment injured the fine flavour, especially in the case of red wines. Then, in 1865, Vergnette-Lamotte (I.) proposed to employ a temperature not exceeding 50° C., thereby avoiding the evil specified without missing the principal object in view; and at the same period Pasteur (XX.), as a result of his researches on wines, found that they could be reliably protected against deterioration by warming them up to 55° to 60° C. With regard to the priority dispute between the two authorities, the reader is referred to the bibliography. Thanks to the reputation at that time enjoyed by Pasteur, the process patented by him was quickly introduced into practice, and called Pasteurisation in his honour.

The inventor's primary intention was to use this process merely for imparting stability to wine, the killing or permanent weakening of the germs therein being effected by the conjoint influence of a temperature of 55° to 60° C. (in itself insufficient for the purpose) and of the toxic action (at this temperature) of the alcohol and organic acids present. Subsequently the name

was extended to the attempts made, by gentle warming, to weaken or kill the germs in other liquids, *e.g.* milk (§ 128), wherein these chemical auxiliaries are lacking. This important difference has not been sufficiently appreciated in the literature, and is not infrequently ignored in practice, the consequence being failures and illusory results. So far as concerns the pasteurisation of wine, beer, and must, which is all that we have to consider in this place, the technology of the process (which is practised in a variety of modifications), must be dismissed in a few words, the reader being referred, for further information, to the descriptions given by Pasteur (XIV.), Babo and Mach (I.), and F. Malvezin (I.). The forms of apparatus latterly constructed for the pasteurisation of wine have been described by Vassillière, Charvet and U. Gayon (I.), the last named of whom (I.) has also made comparative examinations of several systems. Illustrations and descriptions of the apparatus designed for pasteurising (or as it is often erroneously styled, sterilising) bottled beer, will be found in Fassbender's "Technologie," and in the handbooks mentioned in § 81.

The difficulties encountered in the practical pasteurisation of wine, beer and must are mainly of three kinds. First is the selection of the lowest temperature at which it is possible to attain the end in view, viz. to kill the yeast and bacteria, or render them incapable of setting up any further action in the liquid in question. This temperature, however, necessarily varies according to the species of the organisms present, and the chemical composition of the wine or beer. Hence no invariably applicable figure can be given, though 50° to 60° C. may usually be adhered to. The further this limit is exceeded in the direction of the boiling point, the more decided will be the boiled—or in the case of beer, the so-called bread-like—flavour of the liquid treated. This flavour largely owes its origin to the modification of certain constituents of the hot liquid by oxygen. To afford a remedy—and this forms the second difficulty—not only must the access of air be prevented, *i.e.* by warming the liquid in corked bottles or bunged casks, but also the oxygen present in solution has to be eliminated—as was proposed in H. Gronwald's German Patent, No. 98,584 (1896). The third difficulty, which we need not now consider, resides in the formation of a coagulum produced by the heat, and constituting a deposit which, since it would spoil the appearance of the wine when poured out, has eventually to be removed by filtration, followed by a second pasteurisation.

Some very tenacious bacteria are able to survive the pasteurisation of the three liquids mentioned, but are scarcely capable of doing any subsequent damage. Thus, in a sample of Munich beer sufficiently pasteurised for the export trade, Dœmens (I.) found 34 bacteria per c.c., all able to develop on nutrient

gelatin. It may be remarked that the *Mycoderma* are very susceptible to this treatment; and it has been proved that, under the conditions prevailing in the pasteurisation of beer, a temperature of 60° to 65° C. is fatal to six different species, including *Mycoderma rubrum* and *M. humuli;* as also to *Sacch. Past. III.* and several species of beer yeast (Lasché (V.)). In the case of several species of mucinous yeasts examined by Richard Meissner (II.), the limit was found to range between 51° and 61° C. according to the species.

Since it is easier to render the yeasts innocuous than the bacteria, a slighter warming will suffice when there is reason to believe that the latter are entirely absent from, or harmless in, the wine under treatment, and when, consequently, the task is confined to eliminating the yeasts alone, in order to prevent them afterwards setting up secondary fermentation and turbidity. Carl Schulze (I.) has shown that, under these circumstances and in presence of about 10 per cent. of alcohol, the yeasts are killed by exposing the bottled wine to a temperature of 45° C. for two hours. The samples tested in this manner did not exhibit any alteration of flavour, though a precipitation of coagulum occurred.

The preparation of so-called unfermented and non-alcoholic grape and fruit wines, which in reality are nothing more than stabilified grape- or fruit-must—and which, thanks to the temperance movement, are more and more coming into favour—has also benefited by the observations just recorded. H. Mueller-Thurgau (XII.) in particular has occupied himself with this question, and has drawn up practical instructions for pasteurising these beverages, by exposure to a temperature of 60° C. for half-an-hour to an hour. Here also it is necessary to filter off the resulting deposit, and pasteurise again. Fruit juices, *e.g.* the cherry juice so highly appreciated in North America, can also be rendered stable by similar treatment.

The reader will now be in a position to understand and thoroughly appreciate the operation of warming the curd in cheese-making (as mentioned in § 182), and the influence of the temperature then maintained on the subsequent progress of ripening.

CHAPTER XLVII.

ANATOMY OF THE YEAST CELL.

§ 249.—The Cell Membrane.

In the case of younger cells, actively engaged in metabolism, the **cell membrane** is very thin (only a few tenths of a μ) and has nearly the same refractive power as the enclosed plasma. It may be rendered clearly visible and distinguishable by allowing some strongly plasmolytic reagent, *e.g.* dilute acids, to act on the cell, whereby the contents are caused to shrink. The membrane can be exposed in a simpler manner by merely pressing gently on the cover glass of the microscopical preparation, so that the cells burst and the empty membranes become visible as pale skin. The membrane of the permanent cells described in § 245 and § 246 is, however, discernible without this preliminary treatment. According to the researches of H. Will (VIII.), the membrane in these cells attains a thickness of 0.7 to 0.8 μ, or even 1 μ occasionally. This thickening of the cell membrane is a precautionary measure against adverse influences, and occurs more particularly when the yeast cells are obliged to develop in or upon a concentrated nutrient medium, *e.g.* strong ale ("Bockbier") wort or wort-gelatin. C. Becker (I.) determined the thickness of the cell membrane of stock yeast for ordinary Munich beer, as 0.5 μ, whereas that taken from a vat of strong ale measured up to 0.7 μ, and that from the still stronger Salvator beer 0.9 μ. The increase in thickness is accompanied by diminished penetrability, and consequently decreased fermentative power. For this reason the sedimental yeast from strong ales is rarely used again, or only for pitching worts of lower gravity (lager-beer).

The influence of dilute acids (*e.g.* a 1 per cent. solution of osmic acid) or alkalis, on yeast cells, especially the permanent cells, is evidenced by **stratification of the membrane**, which was first observed by H. Will (VIII.) and then confirmed by O. Casagrandi (I.). Mostly the membrane is divided into two layers, or in some instances into three or even more; though C. Becker (I.) never succeeded in finding more than two. The outer of these gradually becomes detached as the cell grows older, a phenomenon first observed by P. Lindner (VI.) in the

case of a Torula. An instructive example of this shedding the membrane is afforded by Fig. 149. The separation (*a*, *b*) may either begin at one spot and then gradually extend to a progressively increased peeling of the outer layer (up to 0.5 μ thick), and the exposure of the inner and more delicate stratum of the membrane; or it may go on simultaneously all over the surface, so that the cell remains enclosed in the unbroken outer layer as in a bag (*c*), through which any daughter-cell that may be produced has to make its way. Apart from such cases the membrane of yeast cells is invariably closed on all sides. It is necessary to emphasise this in view of Bizzozero's (I.) impro-

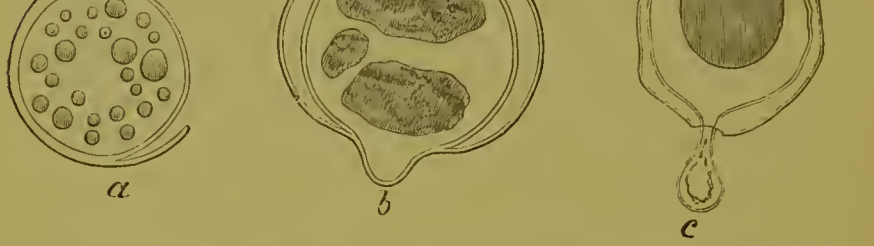


Fig. 149.—Permanent Cells of Bottom-fermentation Beer Yeast.

a. The outer layer of the membrane beginning to peel off. Cell contents rich in drops of oil. From a culture in beer wort.

b. The cell has perished from some unknown cause, before the bud in course of formation had fully developed and separated therefrom. The dead plasma has collected in lumps. The outer layer of the membrane has almost completely loosened, and encloses the cell like the calyx does a flower.

c. A budding cell from which the exterior layer of membrane has become detached on all sides. In this bag, wherein two daughter-cells are just commencing to separate, rests the parent-cell, which is beginning to put forth a third new daughter-cell through a crack in the outer membrane. *b* and *c* were taken from the yeast ring of an old culture in peptone-dextrin-nutrient-salt solution. Magn. 2000. (*After Will.*)

bable report that the membrane of a budding fungus found by him in human epidermis—and of still indefinite position in the botanical system—was full of pores.

More or less perfect fragments of such discarded layers of cell membrane are found in almost every liquid yeast culture of a certain age. There is no danger of their being mistaken, even by a beginner, for the albuminous flakes so common in beer wort cultures, if microchemical reactions be called in aid, since these will quickly show that we have here to do with a substance differing both from albumin and from cellulose.

With regard to the chemical composition of the materials constituting the membrane of the yeast cell, there is not much reliable information to offer at present. Leaving out of consideration what, in the present state of our knowledge, must be regarded as an erroneous counter-opinion expressed by J. Schlossberger (I.), the absence of true cellulose therein was

ascertained by Mulder (IV.), and afterwards by C. von Wisselingh (I.) The absence of any appreciable action of ammoniacal copper oxide on the yeast cell had already been observed by Liebig. According to the concordant results furnished by the researches undertaken by C. von Wisselingh and by Tanret (III.), **chitin**, the second material taking part in the structure of the cell membrane of the *Eumycetes* in general, also seems to be lacking in the case of yeasts. The alleged observation, by Curtis (I.), of a violet colouration produced by zinc iodochloride on the membrane of a pathogenic budding fungus, was declared by Casagrandi to be erroneous; and no more than a faint yellow colouration is produced in the membrane of vegetative yeast cells by simple iodine solution. According to H. Will (IX.), the report made by several observers to the effect that the spore membrane of various yeasts is strongly tinged a yellowish brown by zinc iodochloride, must be corrected in the sense that the seat of this colouration is not in the spore membrane itself, but in an overlying unconsumed residue of the plasma of the parent-cell. This Will was able to determine more particularly in the case of two species of wild yeast examined. According to Lindner (VIII.), the spores of *Schizo-Saccharomyces octosporus* exhibit a very peculiar behaviour in this respect, a powerful blue colouration being imparted by iodine and potassium iodide to the spore membrane, and often to that of the vegetative cells as well. The same behaviour was observed by J. Ch. Holm in another species allied to the one just mentioned. Along with these exceptions must also be classed the blue colouration observed by Liebermann and Bitto (I.) on the addition of zinc iodochloride to a macroscopical preparation of so-called yeast cellulose (§ 225). In connection with this behaviour of the yeast cell membrane towards colouring matters, Casagrandi remarks that Congo Red is not absorbed, whereas, on the other hand, very good staining can be effected with Ehrlich's Methylene Blue or Hanstein's Aniline Violet. C. Becker (I.), however, found only the latter of these two effective. Solution of the membrane occurs on the cells being immersed in concentrated chromic or sulphuric acid, whilst other acids are ineffectual. The same applies to the mixture of nitric acid and potassium chlorate, known as Schulze's maceration liquid, through its mode of action having first been observed and utilised by Brunnengræber in the laboratory of Professor F. F. Schulze at Rostock. This is related by A. Tschirch (IV.) in an admirable historical review of microchemical methods.

The foregoing, together with a series of other microchemical reactions, led Casagrandi to the belief that the yeast cell membrane contains a substance chemically allied to the pectin recognised by Mangin as a constituent of other vegetable membranes. It is, however, difficult to ascertain whether and how

far this view is correct, owing to the absence of means for detecting this carbohydrate. Still more undecided is the question as to the divergent chemical composition of the various strata of the membrane. That some difference does exist may be deduced from the influence exerted by chromic acid (diluted with its own volume of water) on the cell membrane, whereby the inner layer is more readily dissolved than the outer, especially in the case of permanent cells. This observation was first made by H. Will, and was afterwards confirmed by Casagrandi and supplemented by different staining tests. It was probably owing to a similar observation that Nægeli was led to surmise that the cell membrane in the films of Mycoderma is cuticularised.

The hypothesis founded on the microchemical examination of the yeast cell membrane is undeniably supported by the macrochemical researches of Salkowski (VI.) on the same point. He found that the residue obtained by extracting pressed yeast for half-an-hour (§ 254) with boiling 3 per cent. caustic potash solution, and amounting to about 3.1 per cent. by weight of the substance taken, was separable into two components—one soluble, the other insoluble—by prolonged boiling in a large volume of water, under a reflux condenser, or in the autoclave at a pressure of 2 to $2\frac{1}{2}$ atmospheres. On separating and concentrating the solution, the soluble constituent was precipitated by absolute alcohol, as a white powder exhibiting the rotatory power α_D 173.3° to 174.1°. The results of the ultimate analysis indicate the formula $C_6H_{10}O_5$. On hydrolysis with dilute acids, practically the whole is converted into *d*-glucose. Iodine in potassium iodide gives a powerful brown-red stain, on which account the substance has received the name **erythrocellulose**. It differs from the somewhat analogous glycogens in its lower rotatory power. The other constituent of the original residue remains in the form of a colourless jelly at the bottom of the liquid, and, as it is not altered by iodine solution, is called **achroocellulose**. On hydrolysis it furnishes a mixture, chiefly of glucose with comparatively little mannose, and on this account is probably not a uniform substance.

From the above reports one thing seems clear: that the membrane of the yeast cell is a complex structure, both from the anatomical and the chemical point of view. This fact will be regarded as so much the more certain when it is stated that no one has yet succeeded in obtaining the so-called yeast cellulose free from ash constituents and nitrogen. Schlossberger found 1.0 per cent. of ash, and the preparations made by Salkowski contained between 1.7 and 2.6 per cent., whilst Liebermann and Bitto (I.) found 1.8 per cent. in theirs. It does not do, however, to launch out into theories respecting the significance of these inorganic constituents, the more so because the com-

position of this ash is still unknown. Moreover, we have also to reckon with the still uninvestigated possibility that the ash itself is introduced into the membrane during the process of extraction. As regards the amount of nitrogen in the membrane, one is unable to find similar unanimity in the reports of the different workers, for whereas SCHLOSSBERGER (I.) could not reduce it below 0.5 per cent. and E. SALKOWSKI (VI.) arrived at about the same limit (0.4 to 0.5 per cent.), both MULDER, and also NÆGELI and LOEW (II.) claimed to have eliminated it to a trace, though by the use of reagents (*e.g.* strong, warm hydrochloric acid) which few substances could withstand undecomposed.

§ 250.—The Cell Nucleus.

Only in comparatively rare instances is the **cell nucleus** easily recognisable in the living cell without some preliminary treatment, its refractive power being usually only a little greater than that of the **cytoplasm**. In years gone by, when the optical instruments available were inferior in power to those of the present day, the nucleus thus escaped the observation of even practised microscopists. Whereas Nægeli, as long ago as 1844, frequently remarked in the cells of wine and beer yeasts "a small nucleus of whitish mucus adjoining the membrane," the presence of which was confirmed by M. J. SCHLEIDEN (I.) in 1849, ERNST BRUECKE (I.) on the other hand asserted in 1861 that he had never succeeded in finding the same. The earliest important advance was made towards the close of the eighties, by the application, to the botanical examination of cells, of the microchemical staining processes already successfully used in the domain of zoology. FR. SCHMITZ (I.) in 1879 prepared yeast cells by hardening them in a saturated solution of picric acid, then washed them with care, and stained them by immersion in hæmatoxylin, followed by repeated washing. In this way he succeeded in discovering in each cell a small blue-stained body, which was clearly distinguishable from the surrounding colourless plasma, and, both in this and several other respects, behaved very like the nucleus of the animal cell. Strasburger, in repeating the tests several years later, descanted on the somewhat difficult performance of this staining process, and it is therefore not surprising that other experimenters of both sexes, *e.g.* FR. KRASSNER (I.) in 1885, and SIDONIA EISENSCHITZ (I.) ten years later, tried it without success. No more fortunate in this respect was JOH. RAUM (II.) who, in 1890, on the basis of his staining tests (mentioned in later paragraphs) expressed the opinion that the cells of yeast are non-nucleated. A similar negative standpoint seems to have

been taken up also by G. Hieronymus (I.) and A. B. Macallum (I.). On the other hand Schmitz's discovery was confirmed by E. Chr. Hansen (XVI.), A. Zalewski (I.), E. Zacharias (I.), H. Mœller (II.), P. A. Dangeard (II.), Fr. A. Janssens (I.), and L. Buscalioni (I.). The first-named Danish worker also stated that he had been able to discern the nuclei in the cells of the films produced by *Saccharomyces Pastorianus I.* and *II.*, and *S. ellipsoideus I.*, in many cases without trouble. Mœller also observed the nucleus as a homogeneous, pale reddish disc, and expresses the opinion that the same has been mistaken for a **vacuole** by many observers. The present author is able to confirm this from his own experience as a teacher, this mistake regarding the cell nucleus being of somewhat frequent occurrence among practitioners.

The method tested by H. Mœller (III.), and recommended by him as the best for rendering the nucleus visible, is a staining process proposed by H. Heidenhain (I.); and Janssens and Leblanc (I.) also recommended the same as being the most reliable. The method of application consists in fixing a cover-glass streak-preparation with a solution of iodine in potassium iodide, and hardening the same in alcohol as recommended by Mœller (II.); then mordanting it in a $2\frac{1}{2}$ per cent. solution of iron alum, and finally staining with a $\frac{1}{2}$ per cent. solution of hæmatoxylin.

The morphological variability of the cell nucleus was already recognised by Schmitz. In young cells the nucleus is **globular**, but flattens with increasing age to a disc of sinuous contour (Zalewski found this elliptical). The dimensions are considerable, the diameter in some instances attaining to one-third that of the cell itself. According to the researches of Buscalioni and Casagrandi (I.) with *Saccharomyces guttulatus*, the dimensions of the nucleus vary with the size of the cells. Except in the case of ascosporulation (to be referred to later on) Janssens and Leblanc (I.) failed to find more than a single nucleus in any yeast cell during their experiments with pure culture.

The first observation in connection with the internal structure of the yeast cell nucleus, namely the occurrence of a **nucleolus**, was made by Zalewski (I.) in 1885. H. Mœller (II.) in 1892 questioned the accuracy of this report, but it was confirmed a year later, almost simultaneously, by Dangeard (II.) and Janssens (I.), and also later by H. Wager (I.), who found that the cell nuclei of yeast exhibit both a membrane and a nucleolus. The latter is of globular shape, is situated about the centre of the nucleus, and measures in diameter up to one-third that of this last named. The space between the membrane and the nucleolus is filled with **karyoplasm**, which, according to the researches of Janssens and Leblanc (confirmed by H. Wager) has a filamentous structure, forming a sort of framework. The

nucleolus is suspended by the threads of karyoplasm. These two Belgian workers also observed that, under certain circumstances, one or more vacuoles may appear in most of Hansen's species of *Saccharomycetes*, as also in *Schizosaccharomyces Pombe* and divers beer yeasts and pressed yeasts, especially when the cells are transferred into a fresh nutrient solution. They have also been observed by Buscalioni in the nuclei of *S. guttulatus*, but are never found in *Sacch. Ludwigii* and *Schizosaccharomyces octosporus*.

A few remarks may here be made as to the division of the nucleus. The reports hitherto made on the form and structure of the nucleus of yeast have always related to cells not actually engaged in reproduction. When, however, this process sets in, a similar division (reproduction) of the hitherto quiescent nucleus also begins. This operation, which has been already mentioned in § 46 and § 219, may proceed in two ways: either the comparatively simple process of direct subdivision, known as **fragmentation** or **amitosis**, or by the indirect division termed **segmentation**, **karyokinesis**, or **mitosis**. In this latter method the two daughter-nuclei are the result of extensive alterations and modifications of the nuclear structure, as may be read in detail in the treatise of A. Zimmermann (II.) already mentioned. So far as yeast cells are concerned, a certain lack of unanimity on this point prevailed for some time among the various observers. Thus the nuclear reproduction accompanying gemmation was regarded as fragmentation by H. Mœller (II.) in 1892, and this opinion was shared by Dangeard (II.) a year later, Buscalioni (I.) taking the same view in 1896, and H. Wager (I.) in 1898. On the other hand, Janssens (I.) in 1893 opined that the nucleus in the budding yeast cell reproduces by karyokinesis. Equally contradictory are the reports on nuclear subdivision during the formation of ascospores. Janssens considered it karyokinetic, but Mœller (IV.) and Buscalioni held it to be fragmentation. The problem was solved in 1898 by the observation of Janssens and Leblanc (I.) that the nuclei of yeast cells, especially *Saccharomyces Ludwigii* and *Schizosaccharomyces octosporus*, are usually reproduced by karyokinesis, but that this process may undergo a more or less extensive simplification and approximation to direct division, more particularly when the cells are kept under comparatively less favourable conditions of existence. Similar intermediate stages between the two extremes, of mitose on the one hand and amitose on the other, have also been recorded in respect of the cell nuclei of higher plants during the past few years.

We are also indebted to the two last-named Belgian workers for a series of observations on the progress of nuclear subdivision. The first thing noticeable in a cell that is about to bud is the disappearance of the nuclear membrane, and the

elongation and separation of the nuclear body into two parts. The two nucleoli, which are still connected by a thin thread, then move towards that part of the cell at which the bud commences to protrude. One of them passes into the embryo daughter-cell, and develops into the nucleus thereof, enveloping itself with a membrane—as does also the other which has remained in the parent-cell. A view of the different stages of this subdivision is given in Fig. 150. During sporulation a similar subdivision occurs. Two daughter-nuclei are first formed, which, of course, remain in one and the same cell, and thereby give rise to the exceptional case, referred to above, of more than one nucleus being present in a yeast cell. Both these nucleoli eventually undergo a second subdivision, the resulting four nuclei then consuming the existing cytoplasm for the purpose of developing into a corresponding number of

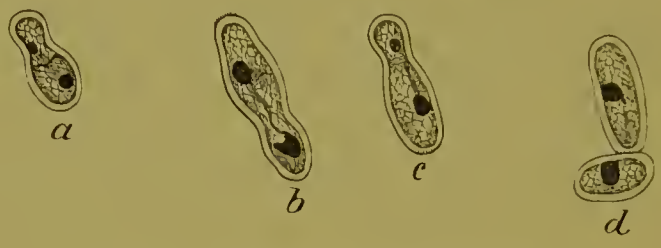


FIG. 150.—Saccharomyces Ludwigii Hansen.

Cells at various stages of budding. Stained.

a, Subdivision of nucleus accomplished; the daughter-nucleus entered into the daughter cell still remains attached to the connecting thread;
b, the latter becomes detached;
c, the septum between mother and daughter-cells commences to form;
d, the separation is complete. Magn. 1200. (*After Janssens and Leblanc.*)

ascospores, each enveloped in a new membrane. In the event of eight spores being formed, the process of subdivision must first be repeated. In cells containing a different number of ascospores (*e.g.* six or nine), it naturally follows that the process of subdivision has not been repeated the same number of times in all the nuclei, but has ceased in some earlier than in others. Finally it may be mentioned that the processes just referred to cannot be distinguished in the living cell without preparation —as KRASSER (II.) unsuccessfully endeavoured to accomplish— but that recourse must be had to a suitable method of staining in order to render them visible.

Since, as already stated, the nucleus in an unstained yeast preparation may readily be mistaken for a vacuole, and *vice versa*, by an unskilled eye, it is necessary to make a few remarks with regard to these vacuoles. As a rule—to which an exception is afforded by *e.g. Saccharomyces apiculatus*—the appearance of one or more vacuoles in the yeast cell is coincident with exhaustion and with a lack of nourishment. Up to the present no

reliable information is available as to the nature of the liquid composing these vacuoles. The refractive power is lower than that of the surrounding plasma, which circumstance alone is sufficient to differentiate them from the ascospores with which they are occasionally confounded by beginners, since a closer examination will quickly show that the refractive power of the latter bodies is (in most cases at least) greater than that of ordinary yeast plasma. This difference becomes particularly apparent when the micrometer screw of the microscope is turned to and fro a little. Under certain circumstances the development of vacuoles (which are mostly globular or oval in shape) may proceed so far that they almost completely fill the cells, the residue of the protoplasm being then reduced to a thin stratum adjacent to the cell walls; or, where two or more vacuoles are present, may form a thin layer between them, and thus, to the inexperienced eye, produce the erroneous impression that a septum has been developed.

FIG. 151.—Crystalloid in the vacuole of a cell of pressed yeast. Near by is a dense cluster of granules. Magn. 4400. (*After Hieronymus.*)

The vacuoles are also occasionally observed to contain **inclusions**. An instance (though a comparatively rare one) of this kind, namely, the appearance of crystals or crystalloid structures, is shown in Fig. 151. Such inclusions have been observed by RAUM (II.) and HIERONYMUS (I.) in the vacuoles of true *Saccharomycetes*, and by H. WILL in those of *Mycoderma* and *Torula*. Janssens and Leblanc, on the contrary, are of opinion that these structures are in reality nucleoli.

A more frequently observed occurrence is the presence, in vacuoles, of a briskly motile body, of which mention will be made at the end of the next paragraph.

§ 251.—The Granules.

In addition to the nucleus, which, as a rule, is not visible until the preparation has been stained, yeast cells generally contain other organised inclusions, which, on the contrary, do not require any artificial aid to become visible, but compel the attention of the observer by their higher refractive power. Since their lustre recalls that of the drops of oil found in the cells of higher plants, *e.g.* in seeds, they have received the name of "**refractive bodies**," or formerly, "**oil drops**." At present they are known as **granules** or **microsomata**. It is only in very young cells that they are not to be found; but they

appear in large numbers at the close of primary fermentation, and are usually very abundant in permanent cells (§ 245). Naturally, the method of nutrition has also some influence on their occurrence. Thus, G. HIERONYMUS (I.) found only very few granules in the cells of pressed yeast that had been grown in a nutrient-salt solution containing grape sugar, whereas they were abundant in such cells as had been kept in milk or a solution of beet sugar. According to a report by JOH. RAUM (II.), no granules are formed when sugar is absent from the nutrient substratum.

It is to the last-named worker that we are indebted for the first accurate investigations in connection with these inclusions. These researches are the more valuable in that they were exclusively performed on pure cultures, namely, of ten species of budding fungi, comprising six true *Saccharomycetes* (*S. cerevisiæ*

FIG. 152.—Permanent Cells
of bottom-fermentation beer yeast, either partially or entirely filled with granules. Cell wall considerably thickened. Magn. 2000. (*After Will.*)

I. Hansen, *S. ellipsoideus I.* and *II. H.*, *S. Pastorianus I. H.* pressed yeast from Warsaw, and a yeast from the air), a red budding fungus termed *S. glutinus*, a so-called black yeast, and a budding fungus from Kephir granules. The occurrence of the inclusions in question was demonstrated in all these species. The number fluctuates considerably, and very often far exceeds a dozen. Their dimensions were also found to vary considerably. When a large number are present, they are comparatively minute (Fig. 152), generally only a few tenths of a micromillimetre in diameter; but when the number is smaller (and it may sink to unity), the size attains considerable proportions. In the latter event they may readily be mistaken, by the inexperienced eye, for ascospores, owing to the high refractive power they have in common with the latter. In doubtful cases the uncertainty may be removed by the aid of some of the microchemical reactions given below. Moreover, these granules are not always of the same shape; for the most part they are globular, though specimens with an angular contour are by no means infrequent. In throwing doubt on the existence of this latter modification, and opining that it only appears on staining,

Janssens and Leblanc (I.) forget that it has also been observed by Hieronymus in the granules of unstained, living yeast cells.

H. Will (VIII.) was the first to publish details concerning the anatomical structure of these granules. Whereas J. Raum had previously failed to detect any structure, and Hieronymus regarded them as crystalloids, Will succeeded in showing, with a series of pure cultures of beer yeasts of divers origin—*Mycoderma*, *Torula*, and species belonging to the *Saccharomyces anomalus* group—that the granules in question consist of at least two distinct constituents: an outer case of albuminous substances and a fat like interior, which latter justifies the name "oil drops" bestowed by Will. The only point on which doubt exists is with regard to the presence of filaments (Fig. 153) which, according to the observations of this worker, extend from the outer case to the interior of the granule and there form a network enclosing the fatty contents of the granule. O. Casagrandi (I.) regards this network as being, not a peculiarity of the unaltered granulum, but as a consequence of the influence of the reagents (*e.g.* alcohol) employed for dissolving out the fatty portions and revealing the alleged reticulations. For the rest, and in its chief points, Will's observation was confirmed by Casagrandi.

Fig. 153.—Permanent Cell of bottom-fermentation beer yeast, containing an unusually large granule. After treatment with alcohol. Magn. 2000. (*After Will.*)

The peculiar behaviour exhibited by the granules towards reagents is easy to understand. With the exception of Raum and Fr. Krasser (II.), all the other observers—including Alb. Klœcker in the case of the so-called *Saccharomyces apiculatus*—are entirely agreed that the granules are turned brown by the action of a 1 per cent. solution of perosmic acid, which is the chief reagent for fats. The fatty contents having been extracted by a suitable solvent, the residual outer case gives all the characteristic reactions for albumin. When yeast cells are treated with concentrated sulphuric acid the membrane swells up; the acid then gains access to the granules and destroys their integument, whereupon the fatty contents of the individual granules coalesce to form larger drops. These turn first green and then bluish-green to blue-black, and, on the addition of a little 10 per cent. caustic potash, are partly or entirely dissolved, *i.e.* vanish from sight. According to Casagrandi's confirmation of the results obtained by Raum, a similar effect is obtained by treating yeast cells with artificial gastric juice: the outer case of the individual granules is digested, whereupon the exposed contents unite to drops, which can then be removed by ether-alcohol.

The action of fat-dissolving reagents on the contents of the still unaltered granules is, as may well be imagined, considerably impeded by the albuminous integument, and requires some time (often far more than twenty-four hours) to attain completion. Lacking the necessary patience for this, one is easily led to the conclusion that the solvent has no influence; as was the case, for instance, with Raum and the ether-alcohol mixture, with Hieronymus and concentrated caustic potash, and with Curtis (I.) and benzene, until Casagrandi, with a greater exercise of patience, showed the contrary. Moreover, it is not surprising that the requisite duration of exposure to these reagents should have been found to vary, not only with the granules of different cells, but also in the case of the different granules in one and the same cell. It is, however, carrying matters to extremes, to do as was proposed by Sidonia Eisenschitz (I.), for example, and attempt to establish a classification of the granules into different groups on the basis of this divergent behaviour.

Fig. 154.—Dead Permanent Cells from bottom-fermentation beer yeast; each containing a large granule which has burst in consequence of the application of pressure to the cover glass. Magn. 2000. (*After Will.*)

From the above-mentioned solubility of the outer cases of the granules in artificial gastric juice, it may be concluded that they are constructed of digestible albuminoids, and consequently that no nuclein is present in the granules. Nevertheless, according to the reports of Fr. Krasser (II.), this rule, which was established by E. Zacharias (I.), has certain exceptions. With regard to the composition of the fat-like contents of the granules, nothing precise can be said at present. Attention should, however, be paid to the reports recorded in the third paragraph from the end of § 253.

The observations (especially those of H. Will) made on the consistence of the granules, show that the fatty contents of these inclusions are not perfectly liquid, but rather semi-fluid. The application of pressure to the cover glass, under which a yeast cell has been placed, causes the granule to burst, as is shown in two examples in Fig. 154.

With regard to the distribution of the granules within the yeast cell, it was found by J. Raum that a certain order prevails, inasmuch as these structures are arranged in series which represent arcs or portions of arcs. Closer investigations, conducted by Hieronymus, supplemented this discovery by showing that the granules are arranged in spirally wound chains, embedded

in a continuous protoplasmic filament, as depicted in Fig. 155. Hieronymus calls this union a central filament. Subsequently, on the basis of the observations of A. Gœrtz, the existence of such an arrangement of the granules was denied by A. Zimmermann (IV.), until Casagrandi confirmed the accuracy of Hieronymus' discovery, with the unimportant limitation that the alleged regularity of arrangement is, in many cases, present to only an inferior extent.

Fig. 155.—Granules in a living cell of pressed yeast in course of budding. Several of the granules are decidedly multiangular, two of them being also much larger than the rest. The two white areas represent vacuoles. Magn. 4400. (*After Hieronymus.*)

Clearly under the influence of Bütschli's theory of the structure of protoplasm (p. 42, vol. i.), Janssens and Leblanc (I.) explained the appearances in question as the knots of the network observed by them in the cytoplasm of the yeast cell (Fig. 156). In the author's opinion, however, it has not yet been strictly proved that these knots are identical with the structures here referred to as granules. The existence of a reticulated framework in the cytoplasm of the yeast cell has, nevertheless (according to a written communication), been also demonstrated by H. Will.

Staining the granules for the purpose of identification can be effected by the method recommended by P. Ernst (II.) and tested by Raum. The preparation is treated with moderately warm (not boiling hot) Lœffler's Methylene Blue solution, followed by washing with water, and finally by staining with a cold solution of Bismarck Brown. This causes the granules to appear black, and to stand well out from the surrounding brown-stained plasma. A differential staining of the integument of the granules can be effected by the method recommended by Casagrandi. Here the yeast cells are fixed (§ 34) with an alcoholic solution of corrosive sublimate, the fatty contents of the granules are then extracted with absolute alcohol, the rest stained with a 20 per cent. solution of

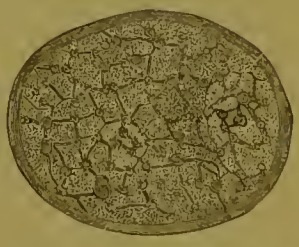


Fig. 156.—Belgian Beer Yeast after a sojourn of forty-four hours in beer wort; observed in the live state. Exhibits the plasmal framework, with appurtenant knots, referred to in the text. The cell nucleus is not depicted. Magn. 2000. (*After Janssens and Leblanc.*)

fuchsine, decolorised, and finally stained by immersion in a 30 per cent. aqueous solution of picric acid. This colours the integument red and causes the granules to stand out clearly from the surrounding plasma, which is stained yellow. Even during the life of the cell, the granules will absorb certain dyestuffs, for instance when, as recommended by Eisenschitz, cells of yeast are cultivated in beer wort containing an addition of a 1 per cent. solution of Benzopurpurin. Some of the granules will stain red by this method in one to two days.

In addition to these granules, which can be detected at once in the unchanged living cell, there are, according to Hieronymus, other similar inclusions, which only become visible after the cells have been fixed with, say, chromic acid or hydrochloric acid. When this treatment has been followed by staining, the "**central filament**" is found to be surrounded by an abundantly looped chain of granules embedded in the portions of the plasma adjoining the wall.

Nearly every one of the workers hitherto named has recorded a certain power of **locomotion** on the part of the structures in question, though the reports on the extent of this power differ. It was remarked by Raum and then confirmed by Hieronymus, that in many instances (though not invariably) a gradual migration of granules from the parent-cell into the young bud goes on during the formation of a daughter-cell. This is represented in Fig. 155. The movement of the granules, however, proceeds very slowly, and bears no comparison with the brisk saltatory motion (Brownian movement?) noticeable in the bodies often observed in the vacuoles of many yeasts, and which are indeed so plentiful in the cells of *Mycoderma* as to constitute a characteristic of this mould fungus. Divergent opinions prevail on the nature of these vacuolous inclusions (so-called saltatory bodies), some regarding them as being invading bacteria, others as endogenous cells, or as dead plasmal excretions. J. Raum was the first to observe the entry of these bodies from the plasma into the vacuole; and it was then shown by E. Kuester (I.) that this migration could be induced by allowing the yeast cells to dry on some substratum. According to the reports of Eisenschitz, a reversal of this migration—namely, from the vacuole back to the surrounding cell plasma, may occasionally take place. The chemical construction of these saltatory bodies can no longer be regarded as identical with that of the plasma granules, since E. Kuester has shown that the two differ in certain respects as regards their behaviour towards dyestuffs. This notwithstanding, neither he nor Symmers (II.) later on, succeeded in arriving at a definite solution of the problem.

In many instances it is observed that the granules offer a greater resistance to decomposition than any other constituents

of the yeast cell, so that, even when the cell membrane has become warped by moisture and the other portions of the cell contents have disappeared from view, the granules remain alone, in the form of free globules (§ 8) as fine as dust, which swim in the nutrient medium, and occasionally render the latter turbid. Probably it is in this sense that we should regard the observations which J. WORTMANN (XII.) had occasion to make in connection with wines that had turned turbid during storage in bottle.

CHAPTER XLVIII.

CHEMISTRY OF THE YEAST CELL.

§ 252.—Chemistry of the Yeast Cell Nucleus.

Recent investigations have shown the probability that, in place of being in a free state in the cell, the **albuminoids** present form part of more highly constituted bodies termed **proteids**. At a given moment these bodies are split up into the albuminous nucleus on the one hand, and the adherent lateral chains on the other. These latter have been termed prosthetic groups by A. Kossel (V.), and explained as the real means by which the vital activity of the cell is enabled to act. Of the groups into which the proteids themselves have been classified, one, namely that of the **nucleins**, is of particular importance, since, as the name itself implies, it furnishes important structural material for the nucleus. A little fuller information on this point will probably be not unwelcome to the reader, and for this reason a few remarks concerning certain conclusions of a practical nature will now be given.

In 1869 F. Miescher (I.) isolated, from the nuclei of pus cells, a nitrogenous constituent to which he gave the name **nuclein**. This body differed from other proteids, both as regards its percentage of phosphorus and by its power of withstanding pepsine. The occurrence of such nuclein in yeast cells was then discovered by F. Hoppe-Seyler (IV.). After doubt had been cast by Nægeli and Loew (II.) on the accuracy of these observations, A. Kossel (III.) succeeded in obtaining considerable quantities of fairly pure nuclein from pressed yeast. For this purpose the yeast, stirred to a pulp, was left for several hours under water, the latter being renewed once or twice in the interim. The yeast sediment was then introduced into dilute caustic soda, which extracted the nuclein. As, however, the latter was at the same time gradually attacked by the solvent, it was found desirable to place the mixture at once on a number of filters and to allow the filtrate to drop into dilute hydrochloric acid, which re-precipitated the dissolved nuclein. The precipitates were then united on a filter, washed with dilute hydrochloric acid and alcohol, and repeatedly extracted with the same, thus furnishing a product which, when dried under the

air-pump, formed a white and fairly pure preparation. Analogous substances have been separated from the most divergent animal and vegetable organs, and classified under the collective term nucleins.

The first investigations concerning the percentage proportion of nuclein to the total nitrogenous constituents of yeast were performed by A. Stutzer (I.). According to this worker, the dried residue obtained from beer yeast by several days' cold extraction with 95 per cent. alcohol followed by drying over sulphuric acid, contained 8.65 per cent. of total nitrogen, of which 2.26 per cent. (or more than a quarter) was in the form of nuclein. Still richer in this respect was the thallus of a mould fungus of undetermined species, which settled from the air into a nutrient solution containing tartaric acid; since, out of 3.78 per cent. of total nitrogen in the dry residue, 1.54 per cent. (or nearly 41 per cent. of the whole) was present as nuclein.

It is to the labours of A. Kossel and his pupils that we are mainly indebted for information on the chemical constitution of nuclein in general, and of yeast nuclein in particular. Their discoveries furnished the basis for Kossel's (II.) classification of the nucleins into two groups. The first includes substances, which, so far as is known, do not occur in cell nuclei, and which were named **paranucleins** by Kossel, or **pseudonucleins** by Hammarsten (III.). When decomposed by dilute acids they yield only phosphoric acid and albumin. On the other hand, under the same treatment, the true nucleins, which alone form the subject of the following lines, furnish, in addition, basic substances to which the name nuclein bases has been given.

An important elucidation of the constitution of nuclein was first presented by R. Altmann (I.) by the discovery that these proteids are decomposed by dilute alkalis into albumin and a nitrogenous acid, rich in phosphorus, which has received the name of **nucleic acid.** This therefore is the prosthetic side chain in the nuclein complex; and it generally receives an additional appellation indicative of the origin of the nuclein in question. All the varieties of nucleic acid exhibit the characteristic behaviour, when heated with dilute acids, that they suffer decomposition into one or other of the nuclein bases described below. The molecule of nucleic acid is composed of a large number of atoms. Those of phosphorus and nitrogen stand in the ratio 1 : 3 in nearly all the instances known. So far, only two exceptions to this rule have been observed. One of them is the **guanylic acid**, isolated from the nuclein proteid of the pancreas of the ox and more closely examined by I. Bangs (I.), the molecule of which acid contains 5 atoms of nitrogen to each atom of phosphorus. The other is a nucleic acid isolated from the embryo of wheat by Th. B. Osborne and G. F. Campbell (I.), the ratio P : N being in this case 1 : 4.

As regards the composition of the albuminoids resulting from the decomposition of the nucleins, less is known than of the nucleic acids with which they are combined. A. Kossel (III.) was the first to investigate yeast nuclein, and he found that the albuminoid substance obtainable therefrom offered greater resistance to the digestive enzymes (pepsin and trypsin) than the yeast nuclein itself, since about 66 per cent. of the latter, but only 3 per cent. of the former could be brought into solution within twelve hours in the course of a comparative test.

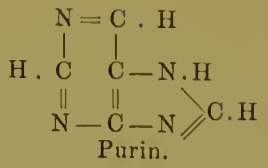
According to A. Kossel (II.), the yeast nucleic acid, first prepared by Altmann, has the formula $C_{17}H_{26}N_6P_2O_{14}$ or $C_{25}H_{36}N_9P_3O_{22}$. The same authority (V.) states that it is decomposed by alkalis into carbohydrates and an acid, namely **plasmic acid**, rich in phosphorus and in nitrogen. The formula ($C_{15}H_{28}N_6P_6O_{30}$) ascribed to this latter acid by Kossel is apparently only approximately correct, since no notice was taken of the iron content (p. 47) subsequently found therein by A. Ascoli (I.). Under the influence of boiling dilute mineral acids, plasmic acid furnishes nuclein bases, together with phosphoric acid and a nitrogenous organic substance which has not yet been more closely examined. A patent has been taken out by K. Schwickerath (I.) for a process of purifying crude nucleic acid from adherent brown mucinous substances, in view of the preparation of yeast nucleic acid on a large scale. In this connection, attention should also be directed to the German Patent, No. 107,734, granted to the Elberfeld Farbenfabriken.

The carbohydrates furnished by the action of boiling dilute mineral acids on yeast nucleic acid (though not from plasmic acid), reduce Fehling's solution, and, according to Kossel (V.), probably consist of a mixture of glucose and one of the pentoses. Liebermann and Bitto (I.) regarded them as an adherent contaminating admixture, of the character of the so-called yeast gum. Similar carbohydrates have also been isolated from various nucleins of animal origin by A. Kossel and A. Neumann (II.), as also by Hammarsten (III.), Alfr. Noll (I.), and by I. Bang (I.). The two first-named workers, however, have also shown that many nucleins contain two kinds of carbohydrates: non-reducing carbohydrates present in the molecule of the nucleic acid; and reducing carbohydrates which, along with the latter substance, take part in the structure of the nuclein complex, and appear in company with nucleic acid on the decomposition of the main substance.

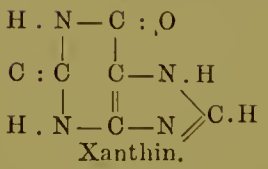
Of the nuclein bases here in question, the first to be discovered in the manner described above was **hypoxanthin** ($C_5H_4N_4O$), which was obtained from yeast nuclein by Kossel (III.) in 1879. Two years later he prepared 10 grams of this base direct from pressed yeast by a method (VII.) of his own,

which he afterwards improved (IX.), and showed (VIII.) that the same is also obtainable from nucleins of different (animal) origin. In the meantime he isolated (III.) from yeast nuclein a second base, **xanthin** ($C_5H_4N_4O_2$), to which he added a third, namely **guanin** ($C_5H_5N_5O$) in 1882 (IX.). The occurrence of the last-named in yeast has also been placed beyond doubt by S. SCHINDLER (I.). These three allies of uric acid ($C_5H_5N_5O_3$) had already been long known to chemists, though not as constituents of nucleins. On the other hand, the four additional bases mentioned below were all new, and were obtained for the first time from the nucleins. **Adenin** ($C_5H_5N_5$) was isolated by KOSSEL (XI.) in 1885, first from the nuclein of ox pancreas and afterwards from pressed yeast. This substance stands in the same relation towards hypoxanthin as guanin does to xanthin, not only as far as chemical composition is concerned, but also in respect of behaviour during putrefaction caused by certain bacteria, which, according to the researches of S. SCHINDLER (I.), degrade guanin to xanthin, and adenin to hypoxanthin. According to A. BAGINSKY (IV.), the last-named offers the greater resistance to the influences in question. In 1893 another new base, namely **thymin**, was brought to our knowledge by A. KOSSEL and A. NEUMANN (I.). It was first obtained from the nucleic acid isolated from the thymoid gland of the calf, which acid received the name of **adenylic acid** on account of its capacity for furnishing adenin. The thymin molecule (which has the formula $C_5H_6N_2O_2$) may be regarded as methyl-dioxy-pyrimidin, in conformity with the views of H. STEUDEL (I.), or as 5-methyl-uracyl, in sympathy with those of E. FISCHER and G. RŒDER (I.), who were the first to prepare it by synthetic means. The hypothesis raised by KOSSEL and NEUMANN (II.) that thymin is also present in the molecular complex of the nucleic acid of yeast, has not been confirmed, though, according to A. ASCOLI (III.), the parent substance of thymin, namely **uracyl** ($C_4H_4N_2O_2$) is found thereiu. It may also be remarked by the way that the fourth of the new nuclein bases, **cytosin**, has so far only been found as a constituent of nucleic acids of animal origin. In the crystalline state this substance has the formula $C_{21}H_{30}N_{16}O_4 \cdot 5H_2O$.

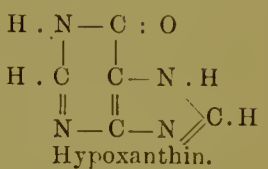
Apart from the last-named, which has not yet been sufficiently investigated, the nuclein bases mentioned may be classified into two groups in accordance with their constitution Adenin, xanthin, hypoxanthin, and guanin are derived from the atomic complex to which EMIL FISCHER (I.) gave the name **Purin** :—



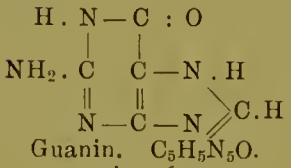
Purin.



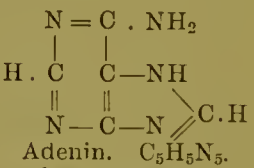
Xanthin.
$C_5H_4N_4O_2$.
2–6–dioxy-purin.



Hypoxanthin.
$C_5H_4N_4O$.
6–monoxy-purin.

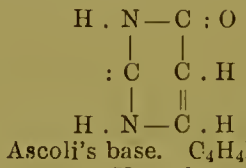


Guanin. $C_5H_5N_5O$.
2–amino–6–oxy-purin.

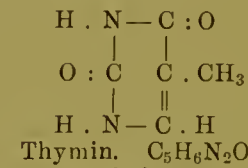


Adenin. $C_5H_5N_5$.
6–amino-purin.

They accordingly belong to the large group of purin bases. On the other hand, Thymin and Ascoli's yeast nuclein base are of more simple construction :—



Ascoli's base. $C_4H_4N_2O_2$.
Uracyl.



Thymin. $C_5H_6N_2O_2$.
5–methyl-uracyl.

Of these, thymin seems the most widely diffused, since only three nucleins not containing this base are known, one of them being yeast nucleic acid. The second is the above-mentioned guanylic acid, which, according to I. Bang (I.), furnishes guanin alone (and of this about 35 per cent. by weight). The third is **inosinic acid**, which was discovered by Liebig in meat juice, and which, according to the experiments made by F. Hasier in 1895, probably contains hypoxanthin solely.

The percentage content of xanthin, guanin, hypoxanthin, and adenin (of course in the combined condition specified) in yeast, was first ascertained by S. Schindler (I.) by the aid of his quantitative method of separation, and was found to be 0.024, 0.029, 0.093, and 0.043 gram respectively per 100 grams of pressed yeast, the character of which was not more accurately defined. These values should not be considered as more than merely approximate, the reliability of analytical methods having been proved doubtful by C. Wulf (I.).

To the reader the situation of nuclein in cell nuclei generally, and in those of the yeast cell in particular, will not appear to have been clearly defined in the foregoing lines. This view is correct. By macrochemical means it is impossible to arrive at more than a hypothesis possessing a certain probability, it being found that cells or agglomerations of cells, containing either a large number of nuclei or nuclei of considerable size, yield a higher proportion of nuclein than such as contain only a few nuclei or those of small dimensions. This was the conclusion formed by Miescher, who succeeded in separating the cell nuclei from the pus cells on which his exhaustive experiments were performed. By similar comparative methods, A. Kossel (I.) came to the conclusion that nuclein, instead of being a reserve material, plays an active part wherever new cells are in course of formation and nuclear reproduction is consequently in progress, *i.e.* in all phenomena of germination. Thus the formation of nuclei and nuclein proceeds simultaneously. This opinion was also shared by H. A. Landwehr (I.). The sole method of obtaining reliable information with regard to the situation of the nuclein within the cell is by microchemical examination. With this object use can be made of the high resistance offered by the nucleins towards pepsin. For example, E. Zacharias (I.) detected the presence of nuclein in the nuclei of pressed yeast cells by the aid of so-called artificial gastric juice (a solution of pepsin in 0.2 per cent. hydrochloric acid). The staining methods, however, are capable of more extensive application.

The various individual chemical constituents of the cell exhibit divergent absorptive affinities for different dyestuffs. It is known that from a mixture of red, blue, or green dyestuffs one cell constituent will absorb chiefly or entirely the first-named, *i.e.* erythrophil, whereas another constituent will prefer the blue (or green), and is therefore termed cyanophil. This preference, however, in each case is determined, not by the shade of colour, but by the chemical constitution (or reaction) of the dyestuff, on the one hand, and by that of the cell constituents with which it is brought into contact, on the other. A cell constituent is therefore said to be **acidophil** when, from a mixture of acid and basic dyestuffs, it absorbs the former and becomes stained therewith; in the converse case it is termed **basophil.** Chemical affinity is almost the sole factor influencing the occurrence or non-occurrence of staining, so that, in presence of a mixture of an acid red dyestuff and a basic blue dyestuff, a given basophil cell constituent will behave as a cyanophil, but, in presence of a mixture of a basic red and an acid blue dyestuff, as an erythrophil. An instance of this kind is afforded by the nucleic acid of yeast, which is strongly basophil, and, according to observations made by H. Malfatti (I.), E. Zacharias (II.), and L. Lilienfeld (I.), is stained (blue or green) by a mixture of Acid Fuchsine

and Methyl Blue (or Methyl Green), but red by a mixture of Safranine and Pale Green. Such behaviour having been macrochemically observed with the known dyestuffs, the same can also be employed microchemically for examining the cell for the presence of one or another chemical substance occurring in local accumulations therein. In any event, care is necessary in the preliminary treatment of the preparation, since in many cases this has a decisive influence on the results. For more detailed information on this point the reader is referred to the monograph by A. Zimmermann (II.). In so far as these microchemical reactions enable a reliable decision to be formed, it has been shown in this manner that the nucleus of the yeast cell is rich in nuclein, and that the latter is probably not present in the cytoplasm. Now, as already shown in § 250, the nucleus consists of the reticulated framework, the sap (or juice), the membrane, and the nucleoli. The first-named, in turn, is composed of the reticulated matter known as **linin**, which is difficult to stain, and of the globules situated at the intersections of the network, these globules consisting of a substance which is readily penetrated by dyestuffs, and which has therefore received the name **chromatin** (§ 35). The nuclein is located in this chromatin, whereas the filaments of linin and the nucleoli contain, in addition to albumin, a substance termed **plastin**, which differs appreciably from, although closely allied to, the nucleins.

The question as to the amount and nature of the other nitrogenous substances associated with the nucleoproteids in the nucleus of the yeast cell, cannot at present be satisfactorily decided, the reports on this matter being few in number and not reliable. The same applies to the nitrogenous constituents of the cytoplasm (§ 219), the scanty communications referring to which will be dealt with in § 255.

The composition of the yeast water prepared in the manner described in § 82 will be briefly considered here from the standpoint of the reports mentioned in the foregoing paragraphs. Even mere boiling in water is sufficient to break up the yeast nuclein, with formation of nuclein bases and free phosphoric acid, which therefore occur as constituents of yeast water. The appearance of phosphoric acid in decoctions of yeast had already been observed by A. Béchamp (VII.), who found, in 1865, that 100 grams of dry yeast lost, on extraction, 2.8 to 3 grams of phosphoric acid, part of which was in the free state. This was confirmed by A. Kossel (III.). He also found that this liberation proceeds rapidly at first, but later on very slowly. With regard to the extraction of nitrogenous substances (xanthin, guanin, sarcin, carnin, leucin, and tyrosin) from yeast cells by the water in which they have been boiled, certain experiments were made in 1874 by P. Schuetzenberger (I.), who, by repeatedly extracting yeast with boiling water

until exhausted, found that, of the 2.78 grams of nitrogen present in 100 grams of fresh yeast (containing 30 per cent. of dry matter), 0 75 gram could be extracted in this manner. In distilling fermented distillery mash, a larger or smaller amount of the nuclein of the contained yeast is similarly decomposed, so that the still residue contains relatively little nuclein, but a large amount of nuclein bases and free phosphoric acid. This fact must be kept in mind, not only when the distillery residue (from potato and grain distilleries) is used as fodder, but also when the same is used as a yeast food (in the manufacture of pressed yeast) and is added in a certain proportion to the mash, a method which is found by experience to result in an increased yield and greater security in working.

A certain practical value, for the foodstuff chemist, attaches to the reports on the decomposition products of yeast nuclein, in cases where it is a question of determining the purity of a given meat extract and detecting the presence of a substitute prepared from yeast. The quantity of beer yeast annually produced in the great brewing countries has been approximately estimated by Feron at about 305,000 tons. Of this amount about 132,000 tons have hitherto been utilised, partly for pitching in breweries and molasses distilleries, and partly as an adulterant of pressed yeast. This leaves still no less than 173,000 tons unutilised; and many brewers have attempted to find a means of putting this waste product to profitable use, instead of letting it run away into the drains or water-courses and thereby leading to constant complaints on the part of neighbours and local authorities. An endeavour has been made to convert this surplus beer yeast, which is rich in proteids and phosphoric acid, into preparations either capable of utilisation as a yeast food in the distillery (especially in molasses distilleries) instead of the usual addition of grain, or else for human consumption as a substitute for meat extracts, or finally as a concentrated cattle food. With this object the yeast is either converted into extract, or is worked up in its entirety alone or with certain adjuncts. A number of processes have been devised for working up yeast into nutritive preparations; and of these, mention may be made of the following for making yeast extracts, viz. those of E. Bauer (II.), Gillhausen, R. Wahl and M. Henius (I.), E. Kressel (I.), Peeters (I.), J. Goodfellow (I.), T. Hill-Jones and E. Kressel (I.), E. Johnson (I.), C. O'Sullivan (I.), D. Watson (I.), A. Denayer (I.), and Lebbin (II.). In the method of G. Eichelbaum (I.), yeast is subjected to the action of species of *Aspergillus* before lixiviation; and according to that of O. G. Overbeck (I.) to the influence of peptase from malt culms. The following methods deal with the yeast as a whole, without extraction: that of Wegener, which is concerned with the production of a coffee

substitute from yeast; that of K. KLEINSCHMIDT (I.), and that of Siebel. According to the latter, a pulp which he calls yeast sugar and which has the appearance and consistence of condensed milk, is prepared by grinding pressed and de-bittered beer yeast with sugar and starch. The preparation of concentrated fodder is the object of the methods of J. STEICKEL (I.) and C. BRUCKER, as well as of the English Patent No. 20,060 of 1893. The purpose of several of the methods first-named above is the recovery of a yeast extract to serve as a substitute for meat extract; and as a matter of fact some of the preparations of this kind already sold are undistinguishable by the laity from real meat extract, so far as the smell, flavour, and external appearance are concerned. Such are, *e.g.* Bios, Eurostose and Carnos, according to A. EICHENGRUEN (I.); and, according to LEBBIN (II.), Ovos as well. An intelligent manufacturer should obviate all chance of such confusion by giving his yeast preparation a special and indubitable title, *e.g.* Vegetable Peptone. This, however, is not always the case, and it would appear that a fraudulent intention is not invariably absent. The foregoing explanations may perhaps divert into a new channel the method of testing adopted by foodstuff chemists for the detection of this yeast extract. By means of the reagents employed in the manufacturing process, and partly also as a result of autofermentation, the nucleins of the yeast suffer a more or less extensive degradation, nuclein bases being liberated. Of the latter, adenin and guanin are found in yeast, and consequently occur in comparatively large amount in the yeast extract. On the other hand, as has been shown by A. KOSSEL (X. and XI.), true meat extract contains little or none of these substances, and is thereby distinguishable from such substitutes. The provision of accurate and reliable data in this respect is reserved for future research in the wide field of the chemistry of the yeast cell.

§ 253.—Glycogen and Fat.

One of the chief phenomena in the metabolism of higher animals is the formation of sugar in the liver. The substance of which this sugar consists was first observed by CLAUDE BERNARD (I.) in 1855, and recognised two years later, under the name "matière glycogène," as a carbohydrate similar to vegetable starch (amylum), dissolving in water to furnish a peculiar opalescent liquid, precipitable by strong alcohol, and giving a brownish-red stain with iodine solution (iodine 2 grams, potassium iodide 6 grams, and water 120). The occurrence of such a colour reaction had been observed four years earlier by R. L. TULASNE (I.) in the young asci of truffles, and a few years later (II.) on those of *Erysiphe Aceris De Candolle*, a species of

the mildew group of fungi. Tulasne seems not to have recollected the observation of his medical colleague, since otherwise he would hardly have contented himself with the briefly expressed hypothesis that a species of albumin with a special affinity for iodine was in question. The next worker in this field, namely A. DE BARY (IV.) in 1863, enlarged our insight into the botanical side of the question by the important discovery that the substance referred to does not appear uniformly distributed throughout the plasma, but is confined to one portion thereof, which he proposed to call the **epiplasma**. At the same time he expressed the opinion that the substance in question was a carbohydrate. Five years later W. KUEHNE (Ia.) reported the occurrence of the "matière glycogène," or glycogen, in *Aethalium septicum*. This discovery, however, did not afford any certain proof of the appearance of glycogen in fungi, the last-named organism belonging to the *Mycetozoa* (§ 23), and therefore merely a connecting link between the fungi and the lower animals. Up to that time no definite proof had been adduced of the existence of glycogen in vegetable cells (those of fungi in particular), and therefore the term "animal starch," applied to this substance, was still in general use.

It was not until 1882 that L. ERRERA (II.), working in A. de Bary's laboratory, showed the principal constituent of fungoid epiplasm to be a carbohydrate, agreeing with animal glycogen in all the properties examined by microchemical means. He also demonstrated its wide distribution, not only in the (first examined) class of *Ascomycetes*, but also in the *Basidiomycetes* and the *Mucoraceæ* (III. and IV.). His discoveries were confirmed by several other workers, *e.g.* by KRAFKOFF (I.) and afterwards by E. LAURENT (VI.), who found this carbohydrate also in *Oidium lactis*, in the conidia of *Cladosporium herbarum*, and in various rose yeasts.

Of all fungi the yeasts seem to have been the most accurately examined with regard to their content of glycogen, and wherein the metabolic importance of this carbohydrate has been most clearly recognised. Even for this reason alone, the present appears the most suitable occasion for dealing with this constituent of the fungi. Its microchemical detection presents no difficulties, there being no other known cell constituent that gives a similar deep brown-red coloration in presence of iodine and is therefore capable of being mistaken for glycogen. Even a beginner would not confound the relatively pale yellow furnished, under the same treatment, by the albuminous cell constituents, with the full brown-red given by glycogen. Should, however, any uncertainty prevail it may be dissipated by utilising the observation first made by ERRERA (II.), to the effect that if the preparation be carefully warmed to 60° to 70° C. after the addition of the iodine, the pale yellow due to albumin

will remain, whilst the red-brown will vanish—to reappear in its original intensity on cooling. If the cells of the treated preparation be ruptured by applying pressure to the cover glass, a rapid examination under the microscope will show that the browned constituents quickly dissolve in the surrounding fluid on escaping from the cells, the residual portion exhibiting the customary yellow coloration furnished by albuminoids on treatment with iodine. If present in any considerable amount, the glycogen becomes visible to the microscopist by virtue of its optical properties, even in unstained preparations. In such event it is seen in the cells as a semi-fluid, whitish opalescent mass of strong refractive power. Sometimes it takes up a position in such portions of the cell contents as are nearest the walls; but in others, as recorded by ERRERA (V.), it collects in one place and assumes the shape of a semilunar aggregation.

The separation and recovery of **fungoid glycogen** by macrochemical means was first successfully accomplished by ERRERA (II.), though the quantities obtained were insufficient for the requirements of an accurate macrochemical examination. This difficulty was first overcome by M. CREMER (III.) in 1894, who was then able to show that the glycogen of yeast is identical with that in the liver of animals, and that too, not merely in respect of the properties aforesaid, but also with reference to its resistance to the action of Fehling's solution and its behaviour on hydrolysis by dilute hydrochloric acid (as also by diastase, saliva, and the pancreatic enzyme), dextrose being produced. E. SALKOWSKI's report (IV.) that yeast glycogen and liver glycogen differ, inasmuch as the former is partly converted into cellulose on being heated to 130° C. along with a little water, was not confirmed by CREMER (III). At all events, the optical rotatory power of yeast glycogen was found to be lower ($a_D = +198.9°$) than that of animal glycogen, for which the values in the literature differ and are in part considerably higher (up to 235°).

For comprehensive researches into the chemistry of this carbohydrate we are indebted to a pupil of Errera's, namely G. CLAUTRIAU (I.), to whose work the interested reader is referred, especially as regards the choice of the best method of isolating glycogen from the different species of fungi. Glycogen is amorphous, and therefore cannot be prepared in the pure state by the crystallisation process, but contains an admixture—larger or smaller in accordance with the origin and care bestowed on the manufacture—of other organic and inorganic cell constituents. This possibility should be kept in mind when mention is made of small discrepancies, similar to those found in animal glycogen by earlier workers, and also by Clautriau in comparing yeast glycogen with that obtained from other fungi. Thus the angle of rotation found by this worker, in the case of yeast glycogen, was 184.5°, whereas in that from *Amanita muscaria*

it was 196.2°. The same applies to the temperature at which the iodine staining disappears; in the case of glycogen from yeast a temperature of 72° to 73° C. is required, whilst in that from other fungi (such as *Boletus edulis*, &c.), decoloration occurs at 58° to 60° C. There is always the possibility of having to reckon with the existence of several isomeric glycogens, in regard to which see an observation by R. Braun (I.).

The quantitative determination of the glycogen content by the Kuelz method—which, as modified by E. Pflueger (II.), furnishes reliable results—is a very troublesome task. Clautriau worked out a colorimetric method, the chief features of which consist in dissolving out the glycogen from a weighed (dry) sample, by repeated extraction with alkaline water, then converting the same into its brown iodine compound, and finally estimating the amount by comparing the colour with that obtained by applying the same treatment to a glycogen solution of known strength. In this manner he obtained the following figures, referred to dry residue: 20 per cent. of glycogen in a sample of *Boletus edulis*; 14 per cent. in a sample of *Amanita muscaria*, and 31 per cent. in a sample of yeast. This last figure agrees with that obtained several years earlier in a different manner by E. Laurent (VIII.), who found that the richest of several yeast samples contained about 32.6 per cent. of glycogen.

On the instigation of L. Errera (VII.), Laurent also endeavoured to investigate the conditions favouring the enrichment of yeast cells in glycogen, and found that, for this purpose, the method of culture on wort gelatin is particularly useful. The following substances were recognised as glycogen formers: lactic acid, succinic acid, malic acid, asparagin, glutamine, egg albumin, peptone, mannite, glucose, lævulose, saccharose, and maltose. According to M. Cremer (I.), these must be supplemented by *d*-galactose and *d*-mannose; but, on the other hand, he found arabinose, rhamnose, sorbose, as well as lactose and glycerin, to be unsuitable; though a contrary opinion with regard to the two last was expressed by E. Laurent. E. Kayser and E. Boullanger (I.) then investigated the influence of other conditions, *e.g.* the presence of air, and the percentage content, of tartaric acid, malic acid, or citric acid, on the inception and progress of glycogen enrichment. In twenty-eight stocks of wine yeast examined with this object, Richard Meissner (III.) found that glycogen makes its appearance, even in the young buds, when the latter have attained about one-fifth the diameter of the parent-cell. A notable observation, and one worthy of further investigation, was made by M. Cremer (VI.), namely, that the glycogen reaction occurred in about twelve hours in a glycogen-free pressed yeast juice that had been treated with 10 per cent. or more of fermentable sugar (dextrose) and kept at

the ordinary temperature. Possibly therefore we must assume the presence of a synthetic enzyme in the cell contents.

In view of the amorphous nature of this carbohydrate, some surprise will be manifested at Laurent's report that the yeast cells are capable of absorbing and accumulating the glycogen present in a nutrient solution. And in fact this assumption was contradicted both by M. Cremer (I.) and by A. Koch and H. Hosæus (I.). The last-named observers found in the case of a pressed yeast, a bottom-fermentation beer yeast, and the so-called Frohberg yeast, that the glycogen added to the nutrient solution (wort or meat extract solution, with or without an addition of grape sugar), whether obtained from calves' liver, rabbits' liver, or pressed yeast, not only remained unutilised, so far as could be ascertained, but also retarded the cell reproduction and fermentative power, so that the yeast crop and the percentage of alcohol in the treated samples were smaller than in those left without addition of glycogen. They failed to detect alcohol in the sugar-free cultures; and concluded that yeast is incapable of secreting a hydrolytic enzyme by means of which the glycogen in the nutrient solution could be converted into fermentable sugar. General application cannot be accorded to this deduction, since M. Cremer (IV.) noted hydrolysis of glycogen on keeping yeast in chloroform water, and found that dextrose was thereby formed. The contrary assumption, by E. Salkowski (II.), of the formation of lævo-rotatory sugar, is not altogether free from objection, as the latter himself (VII.) admitted. Since the conversion of the accumulated glycogen within the cell is probably connected with such an enzyme, a strong presumption exists in favour of the occurrence of a similar enzyme, produced by the plasma. This probability has been brought to almost a certainty by the discovery of Buchner and Rapp (II.) that glycogen can be fermented by expressed yeast juice. The presence of a similar enzyme may also be assumed in other fungi that store up glycogen, and consequently will have to be reckoned with in the separation of this carbohydrate, especially when a quantitative determination of the same is in question. It may be remarked in passing, that, as was first shown by A. Koch and H. Hosæus (I.), the faculty of hydrolysing glycogen is also possessed by various species of bacteria. This at the same time explains Salkowski's (II.) discovery, previously reported by Schuetzenberger and Destrem (I.), as also the results obtained by N. von Chudiakow (I.), and urged by him against the assumption that autofermentation occurs in yeast.

Glycogen has been rightly termed a reserve material. In times of superfluity of nutrient materials the glycogen is stored up in the cell for the purpose of consumption in case of need, either for maintaining the vitality of the individual in an environment destitute of the necessary foodstuff, or for the

construction of new individuals and feeding them until such time as they are able to cater for themselves. This latter event takes place for instance when a sclerotium germinates. In the case of *Coprinus niveus* and *Claviceps purpurea*, L. Errera (VI.) accurately traced the gradual migration of the glycogen from the cells of these hard mycelia into the pileated fungi developed therefrom. He also observed the same in the germination of various fungus spores, *e.g.* those of *Mucoraceæ*. This migration of glycogen is the counterpart of the well-known movement of starch in higher plants, especially during the germination of their seeds. With regard to this worker's report on the formation of glycogen from the oil in the cells, further observations are desirable.

The degradation of glycogen for the generation of energy may either remain only half accomplished, as we shall see in greater detail in a subsequent paragraph dealing with the autofermentation of yeast, or may proceed until the whole is completely consumed into carbon dioxide and water. So far as is known, this condition is reached in the case of yeast cells, when the access of air is unrestricted. Cells of wine yeast that have fallen to the ground, keep alive, thanks mainly to their store of glycogen. It does not always happen, however, that the cells are able to use up this store, since they sometimes perish before the same is exhausted. According to the observations of H. Will (X.), dead permanent cells rich in glycogen can always be found in the sedimental yeast of old cultures in wort or (liquefied) wort gelatin. Finally it may be remarked that the commencement of the working up of accumulated glycogen does not always coincide with the occurrence of a lack of the necessary foodstuffs outside the cell, but seems to be bound up with other circumstances, the age of the cells in particular; so that the glycogen content of a sample of yeast may decline, although sugar is still present in the nutrient solution. In this connection observations have been made by N. Jodlbauer (I.) in the course of some researches which will be referred to in a later section; and these were confirmed by Gontscharuk, whose results have been reported by R. Meissner (III.).

The importance of fats for the life of the yeast cell is equal to that of glycogen; they also are valuable reserve material. Their presence in wine sediment was observed by Braconnot. The percentage of fat varies with the momentary conditions of nutrition of the cells. Payen (II.) fixed it at 2.1 per cent. of the dry residue in the case of beer yeast; but, by the aid of a better method, ensuring complete extraction, Nægeli and Loew (II.) succeeded in separating about 5 per cent. of fat from a bottom-fermentation beer yeast. According to the researches of P. Darexy (I.), alone, and in collaboration with E. Gérard (I.), the chief constituents of yeast fat are stearic

acid and palmitic acid (in almost equal amounts), along with a small quantity of butyric acid, part of the acid being in the free state and part combined with glycerin. The chief seat of the fat is in the granules, whose albuminous membrane prevents lixiviating fluids gaining access to the enclosed fat. Consequently, as remarked by Nægeli and Loew, a simple treatment with ether is ineffectual. Still less likely to effect extraction is the small amount of alcohol present in wine; and, in fact, as shown by Mueller-Thurgau (XVIII.), wine does not contain more than about 0.1 gram of fat per litre. In a case observed by H. Will (VIII.) in 1898, drops of fat were found in both dead and living cells and spores of a ten years old culture of *Sacch. Ludwigii* in beer wort, their colour being reddish-yellow, so that the culture gradually became of a brick-red shade.

A substance closely allied to the fats, namely **lecithin**, which is the cholin ester of palmito-stearo-glycerophosphoric acid, and is therefore constituted in accordance with the formula :—

$$\underset{\text{Palmitic acid.}}{(C_{15}H_{31}COO)}\ \underset{\text{Stearic acid.}}{(C_{17}H_{35}COO)}:\underset{\text{Glycerin.}}{C_3H_5}-O-\ \underset{\text{Phosphoric acid.}}{PO.OH}-\ C-CH_2.CH_2N(CH_3)_3OH\ \text{Cholin.}$$

was first discovered in yeast by Hoppe-Seyler (IV.) in 1866. It was afterwards prepared by him (V.), to the extent of 0.25 gram from 81 grams of air-dry pressed yeast, in the course of rebutting a contrary opinion launched by O. Loew (VIII.) and Nægeli and Loew (II.).

The first discovery of **cholesterin** in fungi was made in 1867, by O. Kohlrausch (I.), in a species of Morel; and soon afterwards it was found by Hoppe-Seyler (IV.) in yeast. Later on, this same worker (V.) recovered 0.44 gram of this monovalent alcohol ($C_{26}H_{43}.OH$) from 81 grams of air-dry pressed yeast. The name cholesterin, which was originally bestowed on a single substance of animal origin, afterwards became enlarged to a collective term, in consequence of the discovery of several isomeric and homologous cholesterins, which, though agreeing with the first cholesterin (from gall, &c.) in their chief properties, yet behaved differently in several reactions. Thus, Hesse isolated from peas the cholesterin to which he gave the name phytosterin; Reinke and Rodewald another (paracholesterin) from *Aethalium septicum;* C. Tanret (IV.) a third (ergosterin), from ergot of rye. According to the researches of E. Gerard (III.–V.), these latter are identical with all the cholesterins hitherto isolated from yeast, as also from *Mucor mucedo*, *Penicillium glaucum*, *Staphylococcus albus*, and indeed from *Cryptogams* generally.

§ 254.—Mucinous Substances of the Carbohydrate Group. The Gelatinous Network.

Under the name yeast gum, several workers have isolated **mucinous carbohydrates** from yeast. Though some of these products have not been closely examined, they have been so far characterised that we may assume the above name to be nothing more than a collective term. They all behave in the same manner towards Fehling's solution, a circumstance of some value in connection with their preparation and isolation, since all are precipitated by this reagent, as a blue, lumpy copper compound, from neutral or faintly alkaline solutions.

On leaving yeast to undergo autofermentation (see a later paragraph) in water, J. Béchamp (VIII.) in 1874, discovered in the liquid (which was protected from invasion by putrefactive organisms) a gummy and optically active substance, the rotatory power of which he ascertained to be $+59-61°$. This substance first received closer attention at the hands of Nægeli and Loew (II.) in 1878, who prepared the **yeast gum** (or **fungoid mucin** as they called it) by extracting yeast with boiling water, freeing the extract from phosphoric acid and peptone with lead acetate, removing the excess of lead with sulphuretted hydrogen, and precipitating the gum with hot alcohol. Repeated solution in water and reprecipitation with alcohol furnished a white powder, which exhibited a relationship with **dextran** in many respects, *e.g.* in being precipitable by alkaline copper solution, though its rotatory power was much less, namely $+78°$. The formula $3(C_6H_{10}O_5)+2H_2O$ was deduced from the results of ultimate analysis. This gum was gradually converted into glucose by the action of acids; was stained brown-red by iodine; and therefore—as Errera (II.) surmised with good reason—also contained glycogen.

Wegner (I.) classed as dextran the gum he obtained in 1890 from yeast by the Scheibler method described in § 160. In the dry state this is a white amorphous neutral powder, which is insoluble in alcohol, and swells up in water to an opalescent liquid, the rotatory power of which, referred to about 1 per cent. strength, was found to approximate to $a_D = +285.7°$.

A **gum (lævulan)**, differing from the foregoing carbohydrates, was obtained by E. Salkowski (II.) in 1889 by precipitating with Fehling's solution a yeast extract, prepared with water containing chloroform for the purpose of preventing putrefaction.

Whilst the undeniable divergences in the results obtained by the above-mentioned workers compel the assumption that different kinds of gum are present in different samples of yeast, the results of Fritz Hessenland's (I.) experiments permit the further conclusion that several kinds of glutinous carbohydrates

may be simultaneously present in one and the same yeast, whether of the top- or bottom-fermentation variety. This worker boiled yeast over a naked flame three times in succession for six hours each, in water containing a small addition of lime, then precipitated with ammonium oxalate the lime from the filtered extract, filtered and concentrated the solution, faintly acidified it with hydrochloric acid, and treated it with an equal volume of 96 per cent. alcohol. The resulting precipitate of brown gum was decolorised by washing with alcohol, and was found to exhibit a percentage composition corresponding to the formula $C_6H_{10}O_5$. The gum from top-fermentation yeast had the rotatory power $\alpha_D = +283.7°$, that of the product from bottom-fermentation yeast being $+287.6°$. The precipitate thrown down from the aqueous solution by Fehling's solution had the composition $2(C_6H_{10}O_5).CuO.H_2O$. This gum furnishes on hydrolysis, not one sugar only but two, namely, a little glucose and a large quantity of *d*-mannose; it therefore chiefly (but not exclusively) consists of **mannan**, the percentage of which, referred to the dry residue of the yeast, amounts to 6–7 per cent.

E. Salkowski (V.) classed as in the main identical with this mannan the yeast gum which he obtained in 1894 by boiling pressed yeast for half-an-hour with a tenfold volume of 3 per cent. caustic potash. This treatment brought into solution the whole of the cell contents, including the gum. When cold this extract was syphoned off from the residue (of so-called yeast cellulose), treated with 15 per cent. (vol.) Fehling's reagent, well mixed and heated. The blue copper compound of the gum separated out in lumps, which were immediately taken out, rinsed with a little water, triturated in a basin with a few drops of hydrochloric acid, and thus converted into a cloudy liquid, from which the gum could be thrown down by a three- to four-fold volume of 96 per cent. alcohol. Repeated solution and re-precipitation finally gave a white, ash-free mass, which was dried with alcohol and ether, and then amounted to about 7 per cent. by weight of the dry residue of the yeast. The results of the ultimate analysis corresponded to the formula $C_{12}H_{22}O_{11}$. In contradistinction to the yeast gum obtained by Nægeli and Loew, this product dissolved readily in water to a filterable, but very glutinous liquid. The sugar furnished by the hydrolysis of this gum seems to have been regarded by Salkowski as *d*-mannose.

Nægeli and Loew thought themselves justified in assuming their yeast gum to be a conversion product of the yeast cell membrane, because they found that fresh quantities of this gum could be obtained from beer yeast by repeated boilings in water, the total amount (including the so-called cellulose) being about 37 per cent. of the dry yeast. It was then found by E. Sal-

KOWSKI (V.) that the residue left after lixiviating yeast with dilute caustic potash yielded no further gum on prolonged boiling; thus proving that, with a more powerful solvent than mere water, the whole of the yeast gum can be extracted at one operation. This, however, does not solve the problem with regard to the location of this mucinous substance; nor at present is any reliable information available as to the seat of the pentosans, which were detected, to the extent of 2 to 3 per cent. (referred to the dry residue) in both top- and bottom-fermentation yeast, by HESSENLAND (I.), with the furfural method.

SCHUETZENBERGER (I.) found in yeast extract a gum, which proved convertible, by hydrolysis, into a reducing sugar. The conclusion that this carbohydrate is an **arabin** is opposed by the further report that the same was converted, by boiling nitric acid, into a mixture of oxalic and mucic acids. From this behaviour it is probable that he had to do with a **galactane**. At all events the amount of carbon found, namely 42.7 per cent., does not correspond with the formula $C_6H_{10}O_5$.

R. MEISSNER (II.) has described a number of budding fungi, which, when sown in wine must, convert the same into an oleaginous ropy liquid, by the aid of a mucinous substance, the composition and method of formation of which are still unknown. Whereas until very recently the ropiness of beer and wine has been attributed almost exclusively to bacterial activity (§ 164), this worker succeeded in showing that this malady can also be induced by budding fungi. He subjected nine such species to accurate examination, several of them having been isolated by himself from ropy wines and the mucinous exudation from plane trees, whilst the remainder were isolated by J. Wortmann as pure cultures from the flora of old bottled wines. They are incapable of ascosporulation or of producing alcohol, and therefore do not belong to the family of true *Saccharomycetes*. In a later paragraph mention will be made of the circumstances under which they become a source of danger.

The gummy substances referred to in the foregoing lines make their appearance in quantity more particularly when the fermentative activity of the cells has drawn to a close. In this event, whether as a result of the swelling of the invariably mucinous outer layer of the membrane, or of an excretion from the interior, the mucinous envelopes developed by the individual cells coalesce to a sort of honeycomb in which the cells appear embedded. These formations were first observed by E. CH. HANSEN (X.), who gave them the name "gelatinous network." They were found in the so-called yeast ring and in the films (§ 246) on old cultures of *Saccharomycetes* and a few other budding fungi, grown in nutrient solutions. They are also not infrequent in the inoculation streaks that have been drawn on

gypsum blocks for the purposes of spore analysis (§ 247); and a similar gelatinous network is often observable in yeast samples (about the size of a pea) that have been taken by the brewer and dried between blotting-paper in order to be conveniently sent by post to a laboratory for examination. Their detection is facilitated by the aid of staining, either with Methyl Violet, which stains the cells only (Fig. 167), or by the so-called capsule staining method (§ 33) described by HANSEN (XVI.). The latter method stains only the mucinous network, and thus renders the latter visible in cases where it could not be detected in the unstained preparation. It sometimes happens that, during the preliminary treatment of the sample of dried cells to be stained,

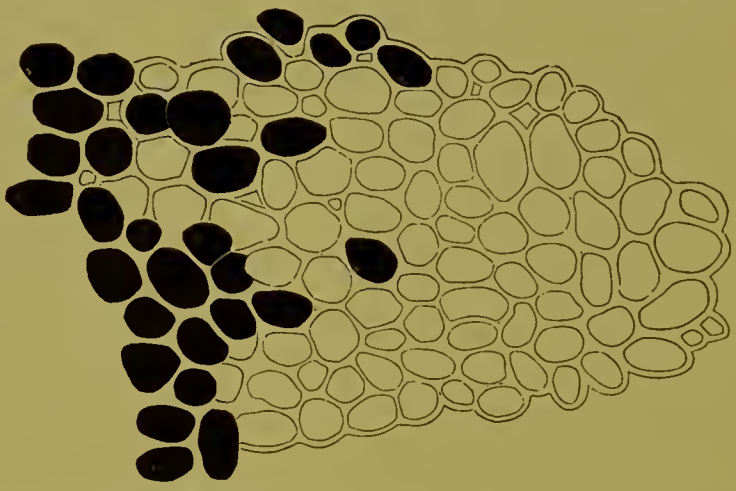


FIG. 157.—Network in Carlsberg Bottom-fermentation Yeast No. 1. Hansen.

The majority of the cells have been washed out of the network in the course of staining with Methyl Violet, only thirty-one cells being left. These, having been deeply stained, have a dense black appearance in the Fig. Magn. 1000. (*After Hansen's original drawings.*)

the cells themselves get washed out of the network, thus leaving the latter isolated and empty. Fragments of evacuated network are not infrequently observed (Fig. 158). Nevertheless, it should not be assumed, without further inquiry, that every formation with this appearance is a residue of this kind; but one must bear in mind the observation made by H. WILL (VII.) to the effect that the glutin bodies present in sedimental yeast frequently exhibit, when dried, an appearance resembling fragments of the network in question. In such cases, decisive information is afforded by an addition of acetic acid to the preparation, this acid dissolving only the remains of the glutin bodies. Will has also shown that several different kinds of reticulation, and substances composing same, are found in the films and yeast ring of wort cultures of beer yeast. This matter will be reverted to in the next paragraph.

The addition of a large quantity of water to the yeast dissolves the network. Nevertheless, as Hansen has shown, it forms anew, unless the treatment be repeated sufficiently often and prolonged. According to H. Will (VII.), however, it is permanently removed when the washing is repeated ten times in twenty-four hours. Now, since the percentage of the constituents forming the network in beer yeast (and also pressed yeast) influences the compressibility of the mass, it follows that this property may under certain circumstances be impaired by the method pursued for washing the yeast (§ 255). On the other hand, it becomes indispensable to remove these mucinous constituents from the yeast when the latter has to be subjected to a quantitative macrochemical analysis for the purpose of ascertaining the composition of the corpus of the cell. This purification might be effected by the washing process, were it

Fig. 158.—Fragments of Gelatinous Network.
From a beer yeast. Unstained. Magn. 1000. (*After Hansen's original drawing.*)

not that the treatment so far modifies the cells themselves as to make the results of the analysis, under certain circumstances, not worth the trouble expended thereon.

In this case also, practical use may be made of the general property of the vegetable gums for thickening to a jelly in presence of borax solution, and thus separating from the liquid. On the basis of an observation by Dumas (III.), H. Will (I.) found that a thick barm of beer yeast, when treated with a 5 per cent. solution of borax, immediately agglomerates into pure white lumps, which quickly subside and unite into large solid masses that fairly crackle when spread out on plates of gypsum. This behaviour is advantageously utilised in the production of pure-culture yeast on a practical scale. The thick barm of yeast from the fermenting cylinder of the pure yeast apparatus is treated with a similar solution of borax, when destined to be sent out in a pressed state. This treatment not only accelerates the deposition of the yeast and considerably facilitates pressing, but also greatly ameliorates the colour of the yeast, and therefore improves its appearace, without impairing the fermentative power to any appreciable extent. It would therefore seem that

the precipitated mucinous matter carries down the yeast cells, but not the brown hop resins, &c.

The foregoing explanations may also prove useful in practical analytical chemistry, namely in cases where the percentage of sugar in wine and similar liquids is to be determined with the aid of Fehling's solution. In such event it should be remembered that this reagent also precipitates the gummy substances present, the resulting precipitate of cuprous oxide also including the precipitated copper compound of the gum; consequently too large an amount of copper is weighed, and the quantity of sugar calculated therefrom will be far in excess of the truth. This applies more especially to the so-called yeast wine, which is an inferior wine obtained by pressing the sediment (the so-called wine yeast) from the wine casks. This sediment is very rich in gum, and therefore (as was shown by K. Amthor (III.)), should not be examined for its sugar content, by the aid of Fehling's solution, without bearing this in mind. With the said yeast wine, in this respect, must be classed the fruit wines (mostly very rich in mucinous matter) and such grape wines as have been racked from the yeast very late, and have therefore absorbed large quantities of gum from the latter during the protracted period of contact therewith. It may also be mentioned that certain gummy substances find their way from the grapes into the wine must, and that experiments have been made by G. Nivière and A. Hubert (I.) with regard to the gum recoverable from wine. Gummy substances are also found in malt, having been isolated therefrom by C. J. Lintner (IV.). It may occasionally happen that such a gum, when not eliminated during mashing and boiling, renders the beer dichroic, instances of which kind have been reported by H. Will (XI.).

§ 255.—Albuminoid Mucinous Substances. "Head" and Frothy Fermentation.

The network enveloping the yeast cells is not invariably composed of the carbohydrate gums described in the preceding paragraphs. Careful investigations performed by H. Will (VII.) have shown that, in the case of a considerable number of stocks of beer yeast, the enveloping, agglomerating network is constructed of a material that gives all the reactions of **albumin**. This albuminous matter is more abundant in the upper layer of the sedimental yeast found in the fermenting tun at the close of primary fermentation, but is less plentiful in the lower or core yeast; and it differs from the other albuminous admixtures present therein, not only in its lack of definite form, but also in point of origin. From the fact that it is also encountered in cultures grown in nutrient solutions free from albumin,

one may conclude that it is elaborated by the yeast cells themselves. Of course the further question must be left undecided whether, in nutrient solutions that already contain albumin, the amount is increased by some portions of this latter substance.

Beer yeast (sedimental yeast) when well shaken up with ether (1:1), takes up a certain quantity of the same, the amount depending on the percentage of this albumin and the aforesaid mucinous substances present. Yeast from the upper layer takes up two to three parts by volume of ether, but core yeast only about one part, the mucinous substances forming bubbles charged with ether. In these laboratory experiments the ether plays the same part as carbon dioxide does in practical fermentation in the vat; and as the mucinous substances do not remain in the sedimental yeast, but are in part ceded to the supernatant liquid, they are able, in the above-described manner, to effect a firmer retention of the carbon dioxide than can be done by mere physical solution. Consequently, under certain circumstances, they may considerably augment the so-called permanent absorptive capacity for carbon dioxide on the part of the beer.

The fact that a soluble albuminoid takes part in the formation of the "head" or scum thrown up by fermenting wort was first suspected by Habich, and then proved, by isolation, by C. Lintner, sen., and Reischauer in 1876. This **froth glutin** (**Krauesenglutin**) is said to originate in the wort or the malt. By means of comparative fermentation experiments with different beer yeasts, it was then established by Alb. Reichard (III.) that, in addition to this malt albuminoid, the presence of certain mucinous excretions (especially those of an albuminoid character) from the yeast is essential to the formation of a normal "head" composed of fine bubbles. The condition of these formative materials in the head at different stages of fermentation has not yet been more closely investigated.

In the case of a liquid absolutely devoid of viscosity and free from mucinous constituents, the bubbles of carbon dioxide liberated in the interior would immediately unite to large ones and make their escape without delay as soon as they arrived at the surface. By means of these mucinous substances, however, each bubble of gas is surrounded by a tough envelope at the instant of liberation, and thereby prevented from becoming merged into others. These bubbles collect on the surface of the liquid, stick together, and thus form by degrees the fine, permanent "head" so desired by the brewer and the pressed-yeast maker. The ascending bubbles also carry to the surface other constituents of the fermenting wort, and incorporate them with the structure of the head, chief among these being yeast cells (see below), then fragments of tissue from the

mashed materials, and finally (in the case of hopped wort), glutin substances and globules of hop resin, to which latter the disagreeable bitter flavour of the head is due. A permanent head of the kind is peculiar to beer, and is not produced in the fermentation of unhopped wort or on wine must; and one must therefore conclude that the presence of hop constituents is indispensable to the structure of a normal head on beer. The accuracy of this conclusion has been rendered more probable by the results furnished by the experiments of E. Ehrich (I.).

The thickness of the head thrown up by the activity of yeast in wort, mash and wine must, is dependent—other conditions being equal—on the available supply of the materials, by means of which the initially naked gas bubbles are converted into mucinous bubbles charged with gas. However, the amount of these substances produced by different stocks of yeast, stands in no definite relation to their fermentative activity, *i.e.* the amount of carbon dioxide liberated in unit time. Hence it will occasionally happen, in comparative fermentation experiments, that a relatively high loss in weight (*i.e.* liberation of carbon dioxide) is noticed in specimens which, from their appearance during fermentation, would seem to have fallen behind considerably. Thus, for instance, in a set of experiments conducted by Mueller-Thurgau (IV.) with 25 stocks of fruit and wine yeasts, the one (a Wædensweil cider yeast) that liberated the largest amount of gas fermented its nutrient substratum without the slightest formation of head. So far, no close attention has been devoted to the capacity of the wine yeasts for depositing albuminoid mucinous substances, though, in addition to the above named, other observations have been recorded indicating the existence of this capacity. Thus, for example, the well-known loss of colour in red wines when treated with white-wine yeasts, seems attributable to the action of similar albuminoids. In contrast to the red-wine yeasts, which are already saturated with colouring matter, the albuminoid mucinous constituents of white-wine yeasts are still in a position to take up tinctorial substances. The case is analogous to that of browned wines when treated with yeast, this procedure, according to Nessler (III.), affording a remedy for the malady in question. The combining and precipitating power of these albuminoid excretions of yeast is probably the cause of the phenomenon—well known in practice, and recommended for utilisation by H. Mueller-Thurgau (III).—that a wine rendered turbid by bacteria can be cured by pitching with a small quantity of must in active fermentation. This must is very rich in yeast cells, Mueller-Thurgau (V.) having detected about 3 milliards per litre. P. Lindner (XVI.) has reported a similar instance of the cure of a sarcina-ropy white beer by adding yeast and rousing.

The chief practical difference between top fermentation and bottom fermentation is due to the aforesaid relation between the yeast cells and the mucinous substances, either produced in the nutrient medium or excreted by the cells themselves. A typical example of top-fermentation yeast in its fullest development is afforded by the stocks forming the chief component of unadulterated pressed yeast. The main features in the preparation of this article by the old or Viennese method are as follow: About three to four hours after the wort has been pitched with yeast—a so-called "artificial" yeast (§ 148) of suitable quality—the mash begins to work. The scum of husks and grains that has accumulated on the surface in the meantime, is now penetrated by an ascending white head, the development and growth of which, during the next twelve hours, presents a picture of ebullient motion of considerable briskness. Under ordinary circumstances the head at the end of this time will have attained a thickness of twelve to fifteen inches, whilst the gravity of the wort will have decreased to about half its initial value. This head forms the vehicle containing the major part of the yeast crop reproduced from the pitching yeast. The bubbles are still transparent. Whereas, during the next three to four hours, the head does not increase in thickness to any great extent, the young cells therein begin to grow; this stage is termed the ripening of the head. The bubbles begin to turn cloudy, and finally reveal the presence of large yeast colonies which, to the unassisted eye, appear as white spots. The reproduction of the cells is now ended; the vat is ripe, to use a practical term; and the gravity of the wort has fallen to about one-third the original strength. The head is next skimmed off by means of suitable skimmers, and is transferred to a sifter in order to separate the coarser adherent particles of the grains, the elimination of the finer undesirable admixtures being effected by the subsequent washing, to which reference will be made in the paragraphs dealing with the autodigestion of yeast. For the present we will merely utter a warning against a one-sided judgment and imperfect appreciation of the importance of the mucinous excretions in connection with facilitating the ascent of the yeast in the fermentation vats during the manufacture of pressed yeast, by calling attention to the influence exercised by the viscosity and chemical constitution of the mash on the working of the yeast, and therefore on the yeast crop. More detailed information on this matter will be found in the volume on pressed-yeast manufacture, issued by O. Durst (I.).

The so-called riotous or bladdery fermentation of beer yeast may be left out of consideration here. The structural materials for the large, tough bladders forming the head in this case are not supplied by the yeast but from the sludge—as was shown

by the researches of A. Reichard (III.). One of the physiological causes determining the appearance of this undesirable phenomenon will be found mentioned in § 258.

Furthermore, only a few words can here be devoted to the so-called frothy fermentation which occurs in spirit distilleries, because the causes of this phenomenon have not yet been sufficiently investigated to furnish results that could be dealt with in the limits of the present work, or permit any reliable conclusions to be drawn. Nevertheless, all the observations on this point recorded in practice may be mentioned as affording noteworthy hints for the more accurate examination of the matter by fermentation physiologists. Special attention may be drawn to the observations recorded by M. Delbrueck (I.). In the normal course of fermentation in potato distilleries, the upper layers of head formed on the surface of the mash disappear in proportion as fresh portions are thrown up from below; whereas, when frothy fermentation occurs, the upper bubbles do not burst, but the thickness of the head goes on increasing, until the entire contents of the vat seem to be in a state of ebullition, and thick masses of froth overflow the top of the vessel. This is a direct source of loss, the more so in countries where the excise regulations prohibit the recovery of the overflow. In many cases the evil may be either entirely remedied, or reduced to a minimum, by pouring a little oil into the contents of the vat as soon as frothy fermentation becomes manifest. Yeasts of high fermentative power are especially liable to this evil, *e.g.* the so-called Race II. already mentioned (page 112); though in other respects this is a very useful yeast. A few observations on this point have been communicated by M. Buecheler (I.), and further information thereon is contained in M. Mærcker's handbook on the manufacture of spirits (I.). Undoubtedly the occurrence of frothy fermentation is determined by special physiological conditions, based on the method of nutrition of the yeast: for example—according to R. Kusserow (I.)—the simultaneous presence of large amounts of amides and peptones in the nutrient substratum. This has been borne in mind in arranging a modification of the mashing process in cases where there is a possibility of this frothy fermentation being set up. According to the method proposed by Hesse, a distillery manager, of Wutzig—as reported by M. Delbrueck (II.)—only one-half of the malt requisite for the saccharification process is introduced into the warm mash, the remainder being added after cooling and the introduction of the pitching yeast. The results of this treatment are reported by G. Heinzelmann (I.) to be satisfactory, almost without exception. In pressed yeast works it also occasionally happens that riotous fermentation is set up in the vats. With regard to the incorrectly named frothy fermentation occasionally met

with in the *masse cuite* and after-products of sugar works without any recognisable cause, and which is regarded by some technicists as a purely chemical process of decomposition, some information will be found in a communication by O. Laxa (I.).

The fact, of which mention has already been made, that certain albuminoid constituents are extracted from yeast cells by ethyl ether, has been utilised in practice by H. Buchner and M. Gruber (I.), who manufacture nutrient preparations therefrom by a patented process.

§ 256.—"Break" and Clarification in Beer and Wine.

In the formation of head on fermenting liquids the aforesaid mucinous excretions from the yeast cell are associated to a greater or smaller extent, according to circumstances, with other mucinous bodies already initially present in the nutrient substratum. Their dependence on such assistance is diminished by the inception of the phenomenon known as "break" in brewing and wine-making.

In proportion as the liberation of carbon dioxide gradually decreases towards the end of fermentation, there ensues a diminution of the force by means of which the yeast cells floating in the fermenting liquid have so far been kept in motion. Consequently the attraction of gravitation can now make its influence on the cells, which have a higher specific gravity than the young beer or new wine. The resulting subsidence of the cells is, however, opposed by two forces, namely, the viscosity of the liquid and the friction between the liquid and the individual cells. These forces suffer diminution when several cells adhere to form a small ball, since, whilst the weight of this agglomeration is equal to the sum of the weights of the individual component cells, the surface is considerably smaller than the total superficial area of the component parts. Hence, while the downward pressure of the weight remains the same as before, the surface of contact with the liquid, and therefore the amount of resistance, is less. Now the aforesaid gummy and albuminous mucinous matters favour, and indeed are indispensable to, this agglomeration of the cells. When they have accomplished this task and united the cells into colonies that are visible to the unassisted eye, the condition known to the brewer as "break" is attained. This condition, which gradually arises towards the end of primary fermentation, forms, in conjunction with the gravity of the wort, the most important factor for determining the ripeness of young beer, and on this account is carefully observed by the brewer. When the process has gone so far that the initially cloudy wort shows up clear in the sampling-glass, with the yeast collected together

into small, gravelly lumps, the brewer is in a position to order the beer to be racked into the storage cask, and thus separated from the sedimental yeast. According as the racking is effected at an earlier or later period, so a larger or smaller number of yeast cells will be carried over into the storage cask; and the beer is said to be racked green or bright respectively. The latter condition is the more desirable, the exact gradation, however, varying according to circumstances.

The progress of clarification in lager-beer, that is to say, its self-purification from the yeast cells, is shown in the following figures, which were determined in 1896 by F. Schœnfeld (I.) in Berlin lager-beer, by means of the yeast counter (§ 83).

One Cubic Millimetre of	Yeast Cells.
Freshly pitched wort contained	14,600
Young beer previous to racking contained . .	54,000
Young beer, racked somewhat green ,, . .	2,000
Young beer, racked bright ,, . .	500
Lager-beer, ready for drinking ,, . .	0.1 to 10

Thus, at the end of the primary fermentation, the number of yeast cells carried over into the storage cask amounts to 5 per cent. when the "break" is fairly good, and to 1 per cent. when the same is very good. Similar results were obtained for North American lager-beer in 1889 by R. Wahl (I.). In order to facilitate the deposition of these yeast cells in the storage cask, it is the custom to place in the cask a number of shavings or strips, either of hazel wood or sheet aluminium. This has been reported on by H. Will (XVIII.). On this foundation the cells adhere firmly, and the microscopical examination of such strips from the empty storage cask shows that the cells are, as it were, glued on to the strips by the mucinous substances already referred to. Imperfectly clarified beer is said to suffer from yeast turbidity or haze, and should not be offered for sale, since, even when the yeast is in small amount, it is often the cause of gastric catarrh, diarrhœa, and indeed slight toxic symptoms, in the consumer. The actual and proximate causes of this action are still undetermined. The literature of this matter will be found collected in a useful work by N. P. Simanowsky (I.) and in a communication by R. Schwanhaueser (I.), a few experiments on the same point having also been undertaken by J. Neumayer (I.). With regard to the clarification of cloudy beer by filtration, reference may be made to § 75 in Vol. i. The proposal made by Otto Reinke (I.) in 1896, to facilitate the clarification of beer by the aid of light, can hardly be recommended, since, as already shown by W. Schultze (I.) in 1888, the agreeable flavour of the beer rapidly disappears under such treatment.

The commencement and development of "break" form the chief criterion whereby the small brewer is able to pass judgment on new stocks of yeast, purchased from outside sources and in use for the first time. This circumstance, it may be remarked in passing, forms the chief reason for the difficulty experienced in introducing pure-culture yeast into the smaller breweries, it being a peculiar feature of many stocks of pure-culture bottom-fermentation yeast that, on their first introduction into the brewery, they exhibit little or no "break" until they have been pitched on two or even three successive batches of wort. Now, it is easy to understand that, in presence of this difficulty, the brewer, being already suspicious of the value of the innovation, should decide to revert to the "good old stock yeast" to which he is accustomed, oblivious for the moment of the many disappointments the latter has already caused him. The causes of imperfect "break" are manifold, and, in part, still undetermined; for instance, an insufficiency of lime (§ 258) in the wort, too low a fermenting temperature, or imperfect rousing during the cultivation in wort. On the other hand, some stocks of yeast exhibit a defective "break" under all conditions, and furnish a thin sedimental yeast instead of a firm deposit in the tun. Despite the greater care involved in working with yeasts of this type, they are still employed, especially when they exhibit other valuable qualities and furnish, for example, a beer characterised by great stability and therefore suitable for export. Such a yeast is met with in the Carlsberg bottom yeast No. 1, by means of which E. Ch. Hansen first introduced his pure-culture method into the fermentation industry. On the other hand, for the production of quick-running ales, it will be found economically advantageous to make use of a yeast that clarifies rapidly.

Yeasts exhibiting coarse "break" and good clarification are still more important in the preparation of champagne than for brewing, since in the former case imperfect clarification cannot be remedied by the use of strips or by filtration. Presupposing a knowledge of the rudiments of champagne-making, it may be recalled that the wine for this purpose is treated with sugar, and is subjected whilst in bottle to a secondary fermentation in order to produce the requisite amount of carbon dioxide. In the old process the fermentation of this sugar was left to the few cells usually present in the wine; the results, however, frequently failed to come up to the expectations formed in this respect. The defect can be remedied by adding a sufficient quantity of a pure-culture yeast to the sugared wine (cuvée), which yeast must of course be specially selected for the end in view. Thus, it must not only possess a high power of resistance towards the presence of a large amount of alcohol in the nutrient substratum, and to a high gas pressure, but also have the pro-

perty of settling down in granular aggregations when fermentation is completed, so that the whole of the yeast separates completely when the bottle is degorged. Now, many of the yeasts present in the wine itself are incapable of fulfilling this last requirement, and for this reason waste has hitherto been inevitable. The yeast refuses to "shake on to the cork" in a satisfactory manner, *i.e.* it cannot be induced to rest in position above the cork by the usual process of carefully tapping and turning the inverted bottles, but adheres in patches on the walls and cannot be dislodged: thus making the champagne of inferior value, or even quite unsaleable. However, thanks to the endeavours of H. Mueller-Thurgau (IV. and V.) and J. Wortmann (V.), champagne manufacturers are now able to obtain pure yeasts which will shake on to the cork properly, and collect as a gravelly deposit without adhering to the sides of the bottle. The removal of the yeast is therefore attended with far less waste of liquor than was formerly the case; and the same may also be reduced to a minimum by means of the process patented in Germany (No. 60,351) by Walfard. In this method the granular deposit of yeast is congealed by immersing the corked bottles in a refrigerating mixture at a temperature of about -20° C.; on the bottle being then set upright, the yeast and cork (which has contracted under the influence of the cold) are easily expelled together by the immediately augmented pressure of the carbon dioxide.

The highest demands on the coherence of the yeast colonies are made on the stocks used for the purposes of the sparkling fermentation introduced into practice by H. Mueller-Thurgau (V.). In order to increase the stimulative effect on the consumer's palate, it has become more and more the custom of late years to make thinner grape and fruit wines, poorer in flavouring extractive substances but rich in carbon dioxide or "sparkling." This enables one to dispense with the artificial enrichment with carbon dioxide (carbonation) already extensively practised (according to Nessler (III.)) in the Moselle district, sparkling fermentation being a more efficient substitute therefor. The clear or clarified wine, which has either been poor in gas from the outset or has become flat through repeated rackings, is treated with an addition of 300 to 500 grams of saccharose per hectolitre, and the whole is then pitched with a pure yeast that remains at the bottom throughout the entire period of gas formation, which soon begins. Contrary to what happens during fermentation in champagne bottles, the fermenting liquid retains its brilliance undiminished all the time, and when finally matured and sparkling can be readily and effectually separated from the settled ferments. Reihlen, of Stuttgart, many years previously arrived at the same result by different means, namely, by the use of what he termed "fettered" yeast, prepared by impreg-

nating fibrous material (*e.g.* wood pulp) with must and sowing it with yeast. When development was complete, he washed off the superficial shells and introduced the "fermentation fibre" (richly interspersed internally with fettered cells of yeast) into the wine cask, where it carried out the desired secondary fermentation without affecting the brightness of the liquid.

END OF VOL. II., PART I.

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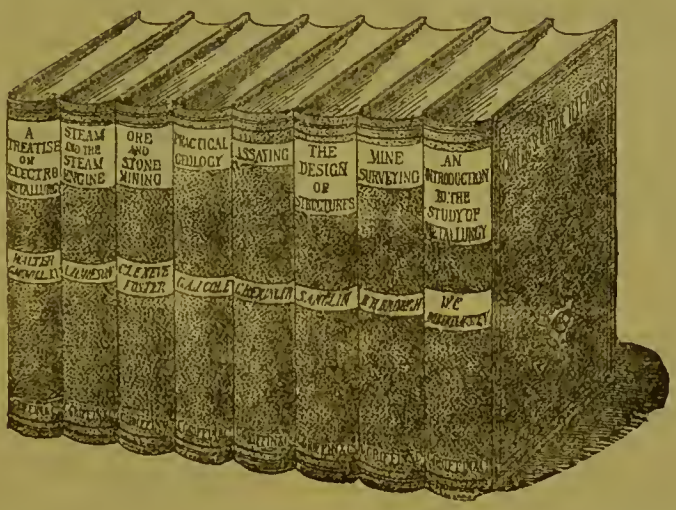

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